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OBSERVATIONS ON POLARITY IN THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM

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In previous work done on the amoeboid slime mold, *Dictyostelium discoideum*, there was good evidence that a chemical substance called acrasin is involved in all its morphogenetic stages. The substance was first demonstrated as responsible for the bringing together of the myxamoebae by chemotaxis in the aggregation stage (Bonner, 1947). Then it was shown that the same substance was continually emitted, in varying amounts depending on the stage of development and the part of the cell mass or pseudoplasmodium, until the final, mature spore-bearing fruiting body was formed (Bonner, 1949). During these later migration and final culmination stages (Raper, 1935, 1940a; Bonner, 1944, for details of the life history) it was shown that there was a high correlation between the amount of acrasin produced by a region and the differentiation of that region into spore or stalk cells (Bonner and Slifkin, 1949).

There are, in the morphogenesis of this slime mold, certain manifestations that can be grouped under the general term polarity, a word which implies a directional quality, a headness and tailness, a symmetry to the developing organism. The question before us (and this paper by no means answers it, but only clarifies the issue to some extent) is what precisely is the relation between polarity and the acrasin mechanism. The mere fact that it is always the anterior end of the migrating pseudoplasmodium that becomes the high region of acrasin emission shows that the two processes are interconnected, but we know little more than this. Polarity is so fundamental and yet so mysterious an aspect of development that any description of some of its activities, which is all I offer here, might ultimately help in guiding us to the real answers.

Observations and Experiments

Vegetative myxamoebae. Polarity in any amoeba presents a special problem, for in most organisms polarity refers to a fixity of direction which is expressed by the fixity of the parts, and in an amoeba there is little fixity of parts. An amoeba may crawl in one direction for a great period of time, but in doing so practically every particle of its substance will pass a moment, however fleeting, at its anterior end, or at any other point. It is true that the nucleus will remain somewhere in

[1] This work was carried out with the help of a grant from the American Cancer Society.

the very general vicinity of the center and also there are occasional evidences of a somewhat more permanent foot or posterior region, but even these phenomena show great variation from moment to moment. And some years ago Jennings (1904) showed, by watching small particles that adhere to the outside surface of an ordinary amoeba, that the surface membrane itself moves in relation to the whole amoeba, by a sort of rolling action.

At any one instant vegetative myxamoebae have a polar appearance, for although their contour is irregular and amorphous, one section of their border (approximately ¼ to ½ of the circumference) is hyaline, while the rest is granular. This is well illustrated in some drawings by Raper (1941). But, just as we have seen in the case of the true amoebae, this polarity is ephemeral.

Aggregating myxamoebae. The myxamoebae themselves, when they commence aggregating, are more elongated than during the vegetative stage, stretching in length to 5 or 6 times their diameter (Bonner, 1947, for photographs of aggregating myxamoebae). But nevertheless, the problems which were mentioned for the vegetative myxamoebae concerning the constant mixing and reshuffling of the internal parts, apply equally well for the elongate aggregating myxamoebae. There is a curious cap on the nucleus which can be seen in stained preparations and with the phase contrast microscope, but even it is not consistently oriented in respect to the aggregation center but is pointed about 50 per cent of the time towards the center and 50 per cent away.

The nearest phenomenon to fixity of parts that is found in aggregating myxamoebae is that the nucleus usually lies in the anterior half of the cell. This is clearly seen in living myxamoebae with an oil immersion phase contrast microscope.[2] The nucleus does, of course, jostle about severely during movement, but its position is most often in the second quarter of the cell from the anterior end. The anterior-most quarter is hyaline and lacks any visible inclusions whatsoever.

Since the aggregating myxamoebae are presumably oriented in their movement by a diffusion gradient of acrasin, it seemed of interest to see what effect suddenly reversing the acrasin gradient might have on them. This was first done in repeated experiments on isolated aggregating myxamoebae and on myxamoebae in thin streams. The center towards which they were moving was simply removed and placed directly behind them. These were done in under-water preparations, using standard solution (Bonner, 1947). Invariably one of two things happened. Occasionally the myxamoebae balled up and after considerable kneading of the surface of the sphere it would send out a pseudopod in the direction of the center at its new location. More frequently the myxamoeba would make a "U" turn without ever losing its elongate shape (see Fig. 1, A).

In another type of experiment a section of a stream of incoming myxamoebae was removed, and reversed. The section, usually about 200 μ long, was cut with a glass needle and turned 180°. If the piece was kept separate from the remaining stream it first contracted and then the individual myxamoebae separated from the

[2] The technique used here was the "thin film of water technique" given in detail by Bonner (1947). The only modification was that the coverslip containing the myxamoebae was placed over a van Tieghem cell filled with light Parke-Davis mineral oil. This greatly enhanced the optical conditions for the phase microscope. Bausch and Lomb phase contrast equipment was used: a 97 × objective, and a long working distance condenser.

piece and moved independently towards the new center (Fig. 1, B). No difference in behavior could be detected between cases where the section was cut and reversed, or merely cut. If the reversed piece was touched to the remaining stump of the stream connected to the center, then they adhered fast to one another. Almost immediately after the operation the reversed section became short and thick and it could be seen that this was because each myxamoeba had become rounded. This rounded appearance of the myxamoebae first reverted to the normal elongated form at the central end of the reversed section as though the myxamoebae had been

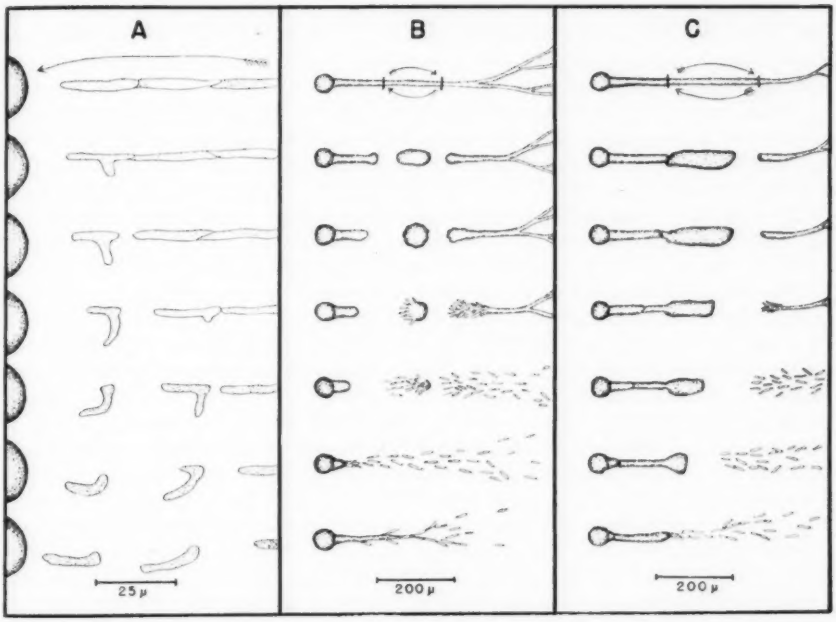

FIGURE 1. Diagram illustrating the experimental reversal of an acrasin gradient during aggregation. A. In the top diagram a center has been removed from in front of the chain of myxamoebae and placed behind. The succeeding drawings show the resulting changes at intervals of 2 min. B. A section of the stream has been reversed (top) and the resulting changes are shown at 4 min. intervals. C. The same as B except that the reversed piece was attached to the proximal stump of the stream.

pulled into their normal elongate shape. In each attempt this passage from the rounded to the elongated form progressed distally until a stream normal in appearance was achieved (Fig. 1, C).

One last curious phenomenon concerning aggregation should be mentioned here and that is the observation of Arndt on aggregation centers that form whirlpools (Arndt, 1937; Raper, 1941). Instead of the amoebae coming directly to a point, they come in at an angle (exactly the way water enters a whirlpool) and the cell mass is not solid, but ring-shaped with a hollow center. Using the under-water

preparations (Bonner, 1947) whirlpool centers often appear and if one watches the ring one can see that the cells follow each other round and round, the ring constantly decreasing in diameter, until the hole in the center disappears; forming finally a solid cell mass. But the important point here is that the cells in the ring move in a polar fashion, even though there is no dominant point, but only a continuous ring.

Migrating pseudoplasmodia. In its external form the migrating pseudoplasmodium is obviously and clearly polar. The anterior end is radially symmetrical and tapers down to a characteristic "bullet-nose," while the posterior end is broad and bilaterally symmetrical, tapering down to a small flat tail. Furthermore the motion shows a fixed polarity and a slime track is deposited posteriorly, that is, it comes off the posterior end.

Previously (Bonner, 1944) serial sections were made of migrating pseudoplasmodia and it was noted that ordinarily there was no marked orientation of the myxamoebae within the pseudoplasmodium except in the anterior portion of late migrating pseudoplasmodia. There the long axis of the cells was at right angles to the long axis of the whole pseudoplasmodium. Upon careful re-examination of the slides it can be seen that the posterior cells occasionally show slight orientation in the antero-posterior direction, most frequently in the cells near the slime sheath. Perpendicular orientation of the anterior cells was also observed in the pre-stalk cells of the tip of the culminating pseudoplasmodium.

A series of studies were made in which the pseudoplasmodia were made to crawl on the underneath side of coverslips, and the cells were observed with the high powers of the microscope (43 × and 97 × objectives). Invariably all the cells that could be seen were actively involved in amoeboid motion continually giving off pseudopodia. With these preparations it was impossible to see the perpendicularly oriented cells in the anterior end, but it was found that if a culminating pseudoplasmodium was placed in a small drop of mineral oil between a slide and a coverslip and the preparation was observed with the phase contrast microscope (43 × objective) the outlines of the cells could be seen.[3] Motion pictures of this were taken with a 16 mm. movie camera at 500 × normal speed, and these showed an even, smooth forward movement towards the tip of all the cells, despite their perpendicular orientation relative to their axis of motion.

It is difficult to reconcile the fact that the long axes of the constituents cells do not always lie in the same direction of the long axis of the whole migrating pseudoplasmodium. Another problem is that since the cells of migrating pseudoplasmodia continue to be pseudopodial, presumably they too (as did the isolated myxamoebae) must have their parts in a continual flux. The puzzling question here is how it is that with each cell in constant turmoil and showing little or no orientation with respect to its movement, that the whole migrating pseudoplasmodium shows polarity of shape and of movement. In an effort to analyze this problem further, experiments attempting to reverse sections of migrating pseudoplasmodia were made.

The first experiments involving reversing sections of migrating pseudoplasmodia

[3] A different species *Polysphondylium violaceum* was used here as they were somewhat easier to handle for this purpose. No difference in respect to the orientation could be observed between it and *Dictyostelium discoideum*.

were done by Raper (1940b). He found that if he cut off a posterior section and replaced it after reversing it 180°, then invariably the pieces crawled away from one another; never fusing and always retaining their original direction of movement. This experiment is easy to perform and I have obtained the same results with many tries.

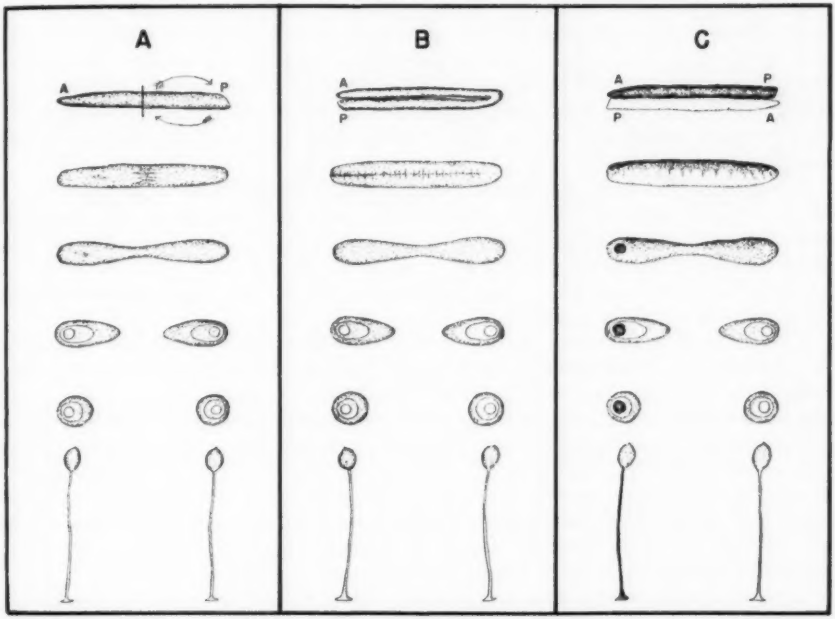

FIGURE 2. Diagram showing the experimental reversal of parts of the migrating pseudoplasmodium. A. The posterior half of a migrating pseudoplasmodium has been reversed and fused carefully onto the anterior half. The changes resulting are given at 15 min. intervals except for the final fruiting bodies drawn at the bottom which have appeared after an interval of 10 hrs. (For convenience the mature fruiting bodies are represented disproportionately small in this figure.) B. A migrating pseudoplasmodium is folded onto itself and the resulting changes are recorded as described for A. C. Two migrating pseudoplasmodia oriented in opposite directions and the upper one stained with nile blue sulphate are carefully fused and the changes are recorded as described for A. Note that the blue and colorless cells intermingle except at the anterior tips.

The point in question here is whether or not, provided the conditions set up in the experiment are just right, a section can be made to reverse if it is under the influence of a high source of acrasin in the close vicinity. One of the possible difficulties encountered in the type of experiment Raper used might be that the slime sheath crowded or herded the cells together, thereby physically preventing them from responding to acrasin. In order to avoid this after a section was reversed, it was gently stirred with an extremely fine glass needle so as to rupture the slime sheath, and carefully fused in this way to the unreversed section. Again the results

were the same, for despite this disruption of the sheath the original polarity of each piece was retained bearing no relation to the overall acrasin emission gradients (Fig. 2, A).

A number of variations of these experiments were undertaken and in each case the object was to cause a reversal of the polarity of a part of the pseudoplasmodium.

In one variation, pseudoplasmodia were folded back so that the two halves touched and the anterior and posterior ends were just opposite each other. The halves were then fused together by passing a fine glass needle back and forth through them. This folded pseudoplasmodium would soon divide into two, forming one center at the "anterior-posterior" end, and another at the "middle" end. These centers were of approximately equal size indicating that the cells retained their original direction of motion, rather than being influenced by the presumably dominant tip region (Fig. 2, B).

In another group of experiments, whole migrating pseudoplasmodia were placed along side each other so that the anterior end of one would lie along side the posterior end of the other. Again they were fused with a needle and again two pseudoplasmodia about equal in size formed at each end of the mass of cells. In some cases the anterior $\frac{1}{10}$ of one of the pseudoplasmodia was removed, but the result of the experiment was the same.[4]

These same experiments were repeated but one of the pseudoplasmodia was stained with nile blue sulphate. Then, after fusion, it was possible to follow the cells of the two pseudoplasmodia. Invariably the two new centers contained both blue and colorless cells, although the center which arose at the site of the anterior end of the blue pseudoplasmodium was predominantly blue and the converse was true of the other center (Fig. 2, C). So we see that although the original polarities of the two pseudoplasmodia were maintained there was considerable mixing of the cells. It was, of course, impossible to ascertain to what extent this was due to the reversing of cells by literally turning them about with the needle, but conceivably this could completely account for the phenomenon.

One final experiment on the migrating pseudoplasmodia should be mentioned, in which pseudoplasmodia were coiled in a ring so that their anterior ends just touched their posterior ends. The two ends were then fused with a needle. After a period of 15 minutes or so, a separation line would appear at its original place and the anterior end would move off in another direction. This would occur even in cases where there was known to be an even acrasin emission the length of the pseudoplasmodium, and in cases where the whole ring was gently stirred with a needle. Apparently the difference between the anterior and posterior ends is sufficient so that their individuality is retained.

Shaking experiments. Using the technique of obtaining myxamoebae for under water preparations (Bonner, 1947), washed myxamoebae in standard solution were placed in 125 cc. Erlenmeyer flasks. The flasks were set on a shaking machine which gently rocked them back and forth. Even though the myxamoebae were at their vegetative stage, after about 5 to 10 minutes of shaking they clumped into

[4] It should be mentioned that in all these experiments, if the disrupting with the needle is sufficiently great, many small centers will arise and therefore care was taken to apply the needle gently.

spherical balls of cells. With continuous shaking for 12 hours these balls of cells became quite polar in appearance, for one end (presumably anterior) was relatively pointed, the other end (posterior) exuded slime sheath material (Fig. 3). It is

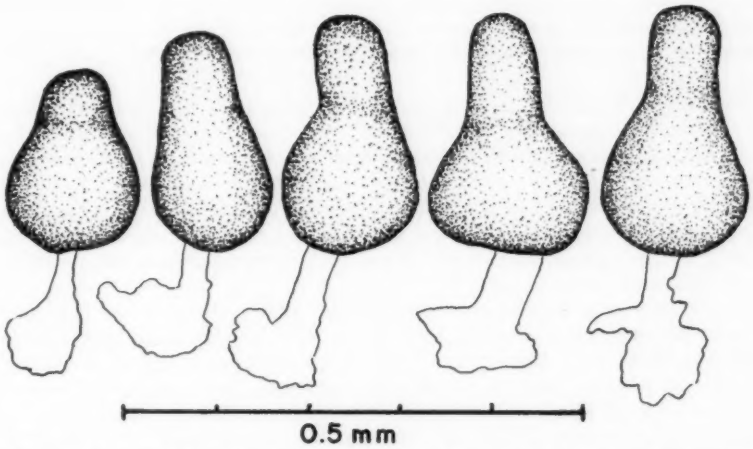

FIGURE 3. Camera lucida drawings of a number of the regular cell masses appearing after 12 hrs. of shaking. Note the slime sheath material issuing from one end. These drawings are of cell masses from one flask and are an example of the most regular forms obtained.

quite remarkable that the over-all polarity becomes evident in a spherical ball of cells under constant agitation. These polar masses were placed among random myxamoebae which oriented towards the mass, indicating that the mass was actively emitting acrasin.

DISCUSSION

One fact that clearly emerges from the above observations is that the over-all, total morphological polarity shows a consistent relation with the polarity of movement, but the smaller constituent parts show little or no relation. In aggregation the external contour of the myxamoebae reflects its polar movement, and also in migration the direction of movement is reflected by the shape of the whole pseudoplasmodium. But in aggregation the parts of the myxamoebae show no such relation (with the possible exception of the position of the nucleus) and in migration, not only the internal parts of the myxamoebae, but also the myxamoebae themselves do not orient with respect to the polarity of the migrating pseudoplasmodium.

It is clear that however the polarity arises in the migrating pseudoplasmodium, it is not necessarily dependent on the polarity acquired during aggregation, for in the shaking experiments it was shown that the polarity could arise in a ball of cells thrown together quickly. The experiments in which the migrating pseudoplasmodia were placed in a ring show that not only is the direction of the movement important, but that there are discreet (although unknown) differences between the

anterior and the posterior end, for these will not fuse but separate from one another after artificial fusion.

In aggregation the gradient of acrasin is capable of governing the polarity, for the myxamoebae invariably orient towards a high concentration of acrasin (Bonner, 1947). However, this is probably not the only factor involved for the adhesiveness of the myxamoebae played a part in the experiment where a section of reversed aggregation stream was attached to another stream and literally pulled into the center (Fig. 1, C).

The best evidence that an external acrasin gradient is not necessarily required for orienting the aggregating myxamoebae comes from the whirlpool configuration, for in that case there is a line of myxamoebae moving in a polar fashion, yet presumably they are all in the same external concentration of acrasin. Even if there were a high point of acrasin production at some region on the ring, then the argument that the acrasin gradient is not orienting the myxamoebae would still hold: for some of the myxamoebae in the ring must be moving away from the region of high acrasin concentration.

In the migrating pseudoplasmodium there is also abundant evidence that an over-all external acrasin gradient does not necessarily govern the polar movement. In the first place, even though the young migrating pseudoplasmodia move about with over-all polarity, they do not possess a gradient of acrasin emission but emit the acrasin equally in all parts (Bonner and Slifkin, 1949). Also, in the experiments in which portions of the pseudoplasmodia were reversed, in no case did the movement of the sections reverse and go towards a high acrasin producing tip (in older pseudoplasmodia the tip produces more acrasin than the remaining portion, Bonner, 1949).

We have then a good demonstration that an over-all acrasin gradient does not account for polar movement or polar shape. There is, unfortunately, no way as yet of determining the distribution of acrasin inside the cell mass, for it is not at all certain that the over-all emission of acrasin reflects its internal distribution at all.

Summary

Experiments designed to give some insight into the nature of polarity were performed on the amoeboid slime mold *Dictyostelium discoideum*. The separate myxamoebae which stream together to form aggregations of myxamoebae show an external polarity and the resulting cell mass has a clear over-all antero-posteriority. In both cases it was shown that the internal parts do not reflect the external polarity. It is known from previous experiments that the aggregating myxamoebae orient in a concentration gradient of a chemical substance tentatively called acrasin, and that acrasin is found in later stages of development. By reversing the gradient of acrasin, separate myxamoebae did not back up but re-oriented towards the point of high concentration. However, it was shown by various experiments that the external acrasin gradient cannot always determine the direction of the polarity, for in the cell masses the polar movement occurs when there is no external gradient of acrasin, or in some cases away from the region of high acrasin emission. It was also shown that polarity can arise in spherical cell masses undergoing gentle shaking in liquid media.

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ALKALINE PHOSPHATASE ACTIVITY IN KIDNEYS OF GLOMERULAR AND AGLOMERULAR MARINE TELEOSTS

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It is generally accepted that glucose undergoes phosphorylation in the process of absorption from the glomerular filtrate (Shannon, 1942). Formation within the tubular cells of a hexose phosphate ester is presumed to create the diffusion gradient which permits a continuous flow of glucose across the lumenal membrane (Kalckar, 1941). Subsequently the ester is hydrolyzed and the glucose discharged into the peritubular blood stream. Presumably phosphorylation is effected at the expense of the labile phosphate bond energy of adenosine triphosphate. In favor of this concept is the presence within the cells of the proximal segments of the renal tubules of rich stores of ATP, hexokinase, and alkaline phosphatase (Kalckar, 1937), the latter two enzymes being necessary, respectively, for phosphorylation of glucose and hydrolysis of the ester. Furthermore, glucose is known to be absorbed solely by cells of the proximal segment (Walker *et al.*, 1941; Walker and Hudson, 1937) which alone contain the enzyme phosphatase in its characteristic location in the brush border (Gomori, 1941). In addition, the absorptive mechanism is an energy consuming one of limited transfer capacity (Shannon and Fischer, 1938) which can be partially or completely blocked by such enzyme inhibitors as cyanide (Bayliss and Lundsgaard, 1928), phlorizin (Lundsgaard, 1933) and mercury (Weston *et al.*, 1949).

Were alkaline phosphatase to be concerned only with the hydrolysis of the hexose ester formed within the tubular cells in the reactions outlined above, it should be absent in those forms which possess no glomeruli, filter no glucose and hence need and presumably have no glucose absorptive mechanism. According to Wilmer (1944) the renal tubules of the toadfish, an aglomerular marine teleost, are devoid of alkaline phosphatase, whereas those of fresh water glomerular teleosts exhibit an abundance of the enzyme. Kalckar (1941) in a similar vein states "aglomerular kidneys (toadfish) contain much less phosphatase than glomerular kidneys from closely related species." If these observations are correct, they constitute strong evidence that renal alkaline phosphatase, at least in the fish kidney, is largely concerned with the absorption of glucose, for the urines of glomerular and aglomerular forms do not differ appreciably in composition (Marshall, 1930).

To test the above hypothesis, the distribution of alkaline phosphatase has been studied in a representative series of marine fish. Because it has been found that the enzyme is present in the brush border of the "proximal segment" of both aglomerular and glomerular forms, it must be concerned with renal tubular functions in addition to, or other than, the absorption of glucose from the glomerular filtrate.

[1] Aided by a grant from the United States Public Health Service.

Methods

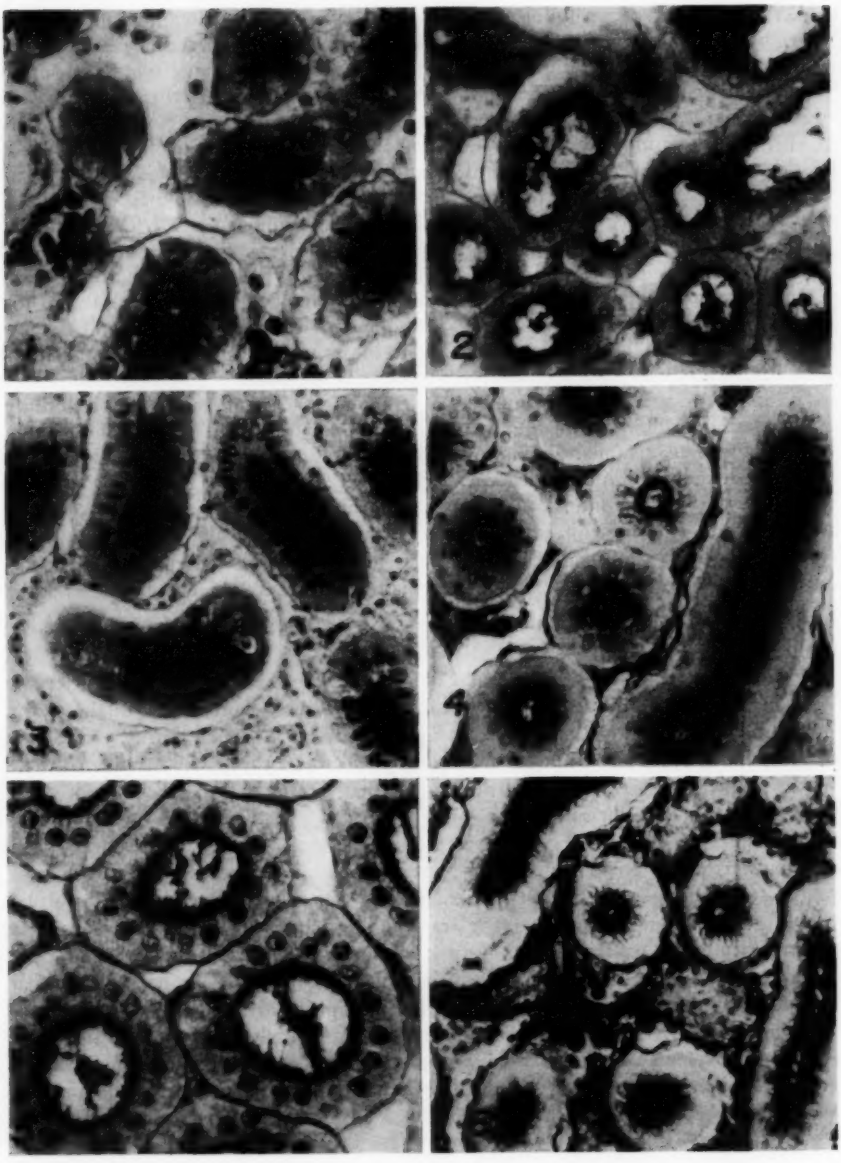
At least three specimens of each of the following fish were caught in traps or seines by the collecting crew of the Marine Biological Laboratory or by commercial dredgers off Menemsha Bight: sea robin (*Prionotus evolans strigatus*), puffer (*Tetraodon maculatus*), eel (*Anguilla rostrata*), dogfish (*Mustelus canis*), skate (*Raja erinacea*), toadfish (*Opsanus tau*), goosefish (*Lophius piscatorius*), and pipefish (*Syngnathus fuscus*). The latter three are aglomerular fish (Marshall, 1934). In addition, specimens of the daddy sculpin (*Myoxycelphalus scorpius*), the long horn sculpin (*Myoxycephalus octodecimspinosus*) and the flounder (*Pseudopleuronectes americanus*) were caught by hook and line at the Mt. Desert Island Biological Laboratory. The daddy sculpin, when full grown, is essentially aglomerular (Grafflin, 1933). All were killed by a blow on the head; the kidneys were promptly removed, and small blocks of tissue were fixed in 95 per cent alcohol for 14 to 20 hours according to the method of Gomori (1939). Further handling, including dehydration and embedding of the tissue in paraffin, was performed as outlined in this method. Sections were cut 6 microns in thickness and those from three to six different species were mounted on a single slide to compare enzymatic activity under identical conditions of treatment. The modified method of Krugelis (1946) was employed to demonstrate the presence of alkaline phosphatase and the slides were subsequently counterstained with light green. Incubation in alkaline glycerophosphate was carried out at 37° C. for ½ to 21 hours. Two types of controls were run: (a) preformed calcium deposits were visualized by cobalt exchange and precipitation by potassium sulfide; (b) the entire incubation and development procedure was carried out, except that glycerophosphate was omitted from the reaction mixture.

Results

Alkaline phosphatase activity could be demonstrated readily in its characteristic location in the brush border of the "proximal" tubular segments of ten of the eleven species of marine fish examined, including all of the aglomerular forms. The only fish in which no activity could be demonstrated in this location, in any one of three specimens, was the eel. In view of the fact that fixation in all blocks from all of these specimens was, for some unknown reason, very poor, we attach no significance to this finding. Plate I, Figures 1 through 6, illustrates in photomicrographs the distribution of the enzyme in six fish: two glomerular teleosts (sculpin and sea robin), two aglomerular teleosts (toadfish and pipefish), one nearly aglomerular teleost (daddy sculpin) [2] and one glomerular selachian (skate). It is apparent that activity is demonstrable in the brush border, in the nuclei of the tubular cells, in the interstitial tissue (so called pseudolymphatic tissue, Marshall and Smith, 1930), in the glomerular capillaries and in some parts of the peritubular network of vessels. Little or none is demonstrable in the cytoplasm of the tubular cells. Preformed deposits of calcium of any magnitude could be demonstrated only in the glomeruli of the eel and in the connective tissue of the renal capsule of the pipefish. When all steps of the procedure were carried out in the usual fashion, except that

[2] This daddy sculpin weighed 570 gms. According to Grafflin (1933), glomerular degeneration should be essentially complete.

PLATE I



glycerophosphate was omitted from the incubation mixture, no precipitate was observed other than that of preformed calcium in the instances noted above.

Discussion

We doubt that quantitative comparisons of enzyme activity (based on incubation time necessary to obtain a given density of precipitate) have, in this material, much cytochemical significance. Although the reaction is without caprice when applied on different occasions to sections from a given block of tissue, sections from different specimens of a given species vary considerably in reactivity. Even sections from different blocks of a given specimen vary somewhat, indicating that minor differences in fixation may account for variability. However, from the limited numbers of specimens in our series, it would appear that the sea robin, skate, pipefish, and toadfish give the most intense brush border reaction. Nevertheless, in one specimen of toadfish it was necessary to incubate for 21 hours at 37° C. to demonstrate enzyme activity adequately. We infer that the failure of Wilmer (1944) and Kalckar (1941) to observe the enzyme in the brush border of the toadfish may be related to inadequacy of fixation, and/or insufficient time of incubation.

Although our results do not in any sense negate the possibility that the alkaline phosphatase of the brush border of the proximal segment of glomerular kidneys functions in the absorption of glucose, they do suggest strongly that the enzyme is concerned in other tubular functions shared by aglomerular and glomerular forms.

Summary

The kidneys of 35 specimens of 11 marine fish have been studied by histochemical methods for the presence and distribution of alkaline phosphatase. All fish examined, with the exception of the eel, exhibited alkaline phosphatase in the brush border of the "proximal" segment. Since the ten species which exhibited the characteristic location of the enzyme included three aglomerular teleosts (toadfish, pipefish, and goosefish), one equivocal aglomerular teleost (daddy sculpin), four glomerular teleosts (sea robin, puffer, flounder, and long horn sculpin), and two

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Plate I

Photomicrographs of sections of the renal tubules of a series of marine fish. All sections 6 micra in thickness; all photomicrographs 650×. Alkaline phosphatase activity was demonstrated by precipitation of black cobaltous sulfide and the sections were subsequently counterstained with light green. Variations in time of incubation (noted below) were used to clarify histological structure and localize activity rather than to quantify the amount of enzyme present. Evidence of alkaline phosphatase activity is seen in the brush border and nuclei of the tubular cells and in the interstitial tissue, but only to a negligible degree in the cytoplasm of the tubular cells. Figures 1 and 2 are from aglomerular fish; Figure 3 is from an essentially aglomerular fish; Figures 4–6 are from glomerular fish.

Figure 1. Renal tubules of the toadfish. Incubation time 4 hours.
Figure 2. Renal tubules of the pipefish. Incubation time 6 hours.
Figure 3. Renal tubules of the daddy sculpin. Incubation time 21 hours.
Figure 4. Renal tubules of the long horn sculpin. Incubation time 2 hours.
Figure 5. Renal tubules of the skate. Incubation time 4 hours.
Figure 6. Renal tubules of the sea robin. Incubation time 1 hour.

glomerular selachians (skate and dogfish), it is concluded that the enzyme of the brush border must be concerned in tubular processes shared by forms with and without glomeruli. It cannot, therefore, function solely in the absorption of glucose from the glomerular filtrate.

The authors are indebted to Miss Stella Zimmer for the preparation of the photomicrographs.

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PHOTOSYNTHESIS AND PHOTOREDUCTION BY THE BLUE GREEN ALGA, SYNECHOCOCCUS ELONGATUS, NÄG.

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The behavior of blue-green algae toward light has been examined by several investigators in the past, and it has been known for some time that these organisms carry out photosynthesis with the evolution of oxygen (Engelmann, 1883). The work of Emerson and Lewis on the participation of phycocyanin in the photosynthesis of *Chroococcus* has again increased the interest in the photosynthetic mechanism of blue-green algae. There are observations in the literature (Nakamura, 1938) which would indicate that certain algae, including several of the blue-greens, are able to oxidize sulfide to sulfur in the light. The interpretation of Nakamura's data is somewhat difficult, and we shall attempt to elucidate certain phases of this problem in a later paper.

Several years ago it was discovered that certain green algae, when incubated in molecular hydrogen, will undergo a change in their metabolic behavior (Gaffron, 1939). In the light these algae will now consume carbon dioxide with the simultaneous uptake of molecular hydrogen while the evolution of oxygen has ceased. To demonstrate this phenomenon, aerobically grown algae have to be incubated for several hours in darkness in an atmosphere of hydrogen before photoreduction of carbon dioxide by hydrogen can be observed in the light. This change of metabolism which involves the activation of a hydrogenase system has been designated as adaptation. It could also be shown that partial pressures of oxygen above 0.5 per cent or high light intensities can bring about de-adaptation, whereupon the organism will return to its normal aerobic metabolism. This work has been reviewed by Gaffron (1944) and by Rabinowitch (1945). Attempts have been made to adapt species of the blue-green alga *Oscillatoria* to such anaerobic conditions, but these experiments proved to be unsuccessful. (Gaffron, unpublished; summarized by Rabinowitch, 1945, p. 129.)

In this paper we shall report on the metabolism of a species of blue-green algae which can be adapted. In the dark and in the presence of molecular hydrogen a hydrogenase becomes active after several hours of incubation. On exposure to low light intensities the organism will absorb carbon dioxide and hydrogen (Table I), and thus behave like certain species of green algae which can be adapted anaerobically.

The first species in which we were able to demonstrate photoreduction was *Synechococcus elongatus* Näg. (Geitler, 1925), and we used a naturally occurring water bloom for our experiments. We have not been able to grow this organism

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and also were not able to find a comparable bloom the following year, and thus we had to discontinue our physiological studies of this species. We were fortunate to find a species of *Chroococcus* which exhibits the same physiological characteristics. This organism has been grown in pure culture and is available for further studies (Frenkel, 1949).

Two other blue-green algae were tested for their ability to carry out photoreduction. *Nostoc muscorum* was very kindly supplied to us by Dr. F. E. Allison, and a species of *Cylindrospermum* by Mr. R. Hecker. We were not able to demonstrate an active hydrogenase in either of these organisms; therefore, it appears that the existence of adaptive hydrogenase systems in algae is not restricted to several species or strains among the green algae, but seems to have a more widespread dis-

TABLE I

Photoreduction by suspensions of Synechococcus elongatus, Näg.

Vessel (1). 4 ml. of 0.1 *M* Warburg bicarbonate-carbonate buffer No. 9 (Na:K = 2:1); gas atmosphere H_2; $k(H_2) = 1.34$

Vessel (2). 4 ml. of *M*/100 bicarbonate solution (Na:K = 2:1); gas atmosphere 4 per cent CO_2 in H_2; $k(CO_2) = 1.68$ (corrected for retention); $k(H_2) = 1.30$; temperature = 25° C.

Treatment		Duration in minutes	Pressure change in mm. per minute		$\frac{-\Delta H_2^*}{-\Delta CO_2}$	Remarks
	Light intensity in foot candles		Vessel (1)	Vessel (2)		
Dark	—	40	− .04	− .09		
Light	20	60	− .37	− .90	0.58	Incomplete adaptation of the hydrogenase system
Light	30	20	− .85	−1.25	1.97	
Light	30	40	−1.05	−1.50	1.90	
Light	30	50	−1.20	−1.53	3.0	
Light	62	10	−1.05	−1.45	3.1	
Light	100	10	− .35	−1.45	0.26	
Light	107	10	− .15	−1.35	0.1	Reversion to normal evolution of oxygen upon continued illumination at this light intensity

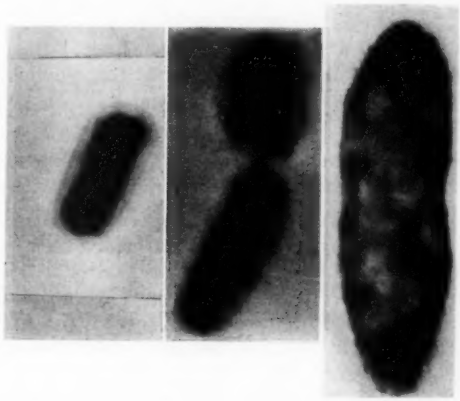
* Calculated from observed pressure changes in vessels (1) and (2).

tribution. Until more is known about the distribution of this enzyme system in other groups of algae and in higher plants, it will be difficult to make any generalizations as to the physiological and evolutionary significance of this anaerobic type of metabolism.

MATERIALS AND METHODS

During the summer of 1947, one of the authors (E. B.) discovered a rather abundant occurrence of unicellular blue-green algae not far from Woods Hole, Massachusetts. These algae occur in small depressions filled with rain water on Angelica Rock, a small island in Buzzards Bay near New Bedford. Angelica Rock is frequented by a great number of gulls and terns, and thus parts of the island

take on an almost white appearance from the droppings of the birds. The exposed rock consists mainly of granite which has weathered considerably and shows a series of small puddles which are about ½ to 3 feet wide, 1 to 15 feet long and ½ to 1½ feet deep. These water areas were almost completely exposed to the sun and the winds with very little protection from the walls of the rock. At the time we made our collection (August 28, 1948, 2:30 P.M.), the temperature of the water in the exposed depressions was 28° C. Some of the smaller depressions gave the appearance of having dried out, others were filled with a dense suspension of blue-green algae which gave a dark green appearance to the water. The green suspension which we collected proved to consist, on the basis of fresh weight, of 1 per cent of blue-green algae and was almost completely free of other organisms. The suspending medium at the time of collection had a pH of 8.65, but contained only traces of bicarbonate and carbonate, also only traces of sodium and chloride.



a. b.

FIGURE 1. Electron micrographs of *Synechococcus elongatus* Näg. 1 a. Air dried cells. ×13,500; b. Cells treated for 24 hours with an aqueous solution of 0.2% osmium tetroxide. ×31,500 (electron microscope magnification × 15,000).

The bottom of the depressions was covered with a layer, several inches deep, of a very dark, loose mud which definitely smelled of hydrogen sulfide and gave a test for sulfide with lead acetate. This mud also contained unicellular blue-green algae as well as abundant protozoa, daphnias, and a variety of other organisms.

The species of *Chroococcus* mentioned previously can readily be grown on agar and in liquid media of similar composition as the mineral medium described by Emerson and Lewis (1942), (Frenkel, unpubl.).

Appearance of the blue-green algae (Fig. 1)

The algae which we collected appear to have no visible sheath as seen under oil immersion; under the electron microscope, however, a very delicate and narrow sheath is revealed. They occur either singly or in a linear row up to 4 cells. The

single cells are 1 to 2 micra wide and from 1½ to 3 micra long. From descriptions given in the literature we are inclined to classify them under *Synechococcus* Nägeli 1849, and according to Geitler would classify them as *Synechococcus elongatus* Näg. These blue-green algae are gram negative and are associated with colorless bacteria which are mostly also gram negative.

Pigmentation

Dr. C. B. Van Niel, to whom we sent samples, pointed out to us the similarity of the visible absorption spectrum of our intact algae with that published for *Chroococcus* (Emerson and Lewis, 1942). On extraction with an 80 per cent acetone-water mixture followed by 100 per cent acetone, all the yellow pigments and the chlorophyll can be readily extracted. The chlorophyll fraction was purified chromatographically and its spectrum corresponded to that of chlorophyll *a*. The blue pigment is difficult to extract. The only way we have been able to obtain a small amount of soluble phycocyanin is by treatment of the algae with 0.1 *N* hydrochloric acid on a steam bath for 10 minutes. The blue pigment thus obtained gives an absorption spectrum with a protein band similar to that published for phycocyanin from blue-green algae by Svedberg and Katsurai (1929). We have found no evidence for the occurrence of Phycoerythrin in *Synechococcus elongatus*.

Measurement of photosynthesis and photoreduction

We have employed the conventional Warburg equipment for the measurement of gas exchange. As a light source we used a bank of 150 watt Mazda bulbs, whose intensity could be varied by means of a rheostat or by changing its distance from the experimental vessels. All measurements were made at 25° C.

To prepare the algae for measurements of gas exchange we first centrifuged the suspension at 100 times gravity for five minutes, which took down only a small fraction of the blue-green algae. The supernatant suspension was decanted and centrifuged at 2000 times gravity for ten minutes. The supernatant was discarded although it was still light green. The sediment was then resuspended in dilute bicarbonate solution or in a dilute bicarbonate-carbonate buffer (Warburg No. 9, Na : K = 2 : 1). The cells were washed once or twice more in these media and then made up to the proper volumes. We collected several gallons of suspension containing *Synechococcus* which were stored in a refrigerator at 3° to 5° C. Under these conditions of storage the algae remained healthy, and we did not observe any decline over a period of two weeks in their capacity to carry out photosynthesis or photoreduction when brought to room temperature.

Results

A storage culture of these blue-green algae, when transferred to a dilute bicarbonate or a bicarbonate-carbonate buffer, will carry out photosynthesis at a constant rate for several days. In phosphate buffer, at pH 5.8 in equilibrium with 4 per cent CO_2 in air, the photosynthetic rate declines quite rapidly with time, and thus we have made all our measurements in bicarbonate solutions or bicarbonate-carbonate

buffers. At light saturation (approximately 1000 foot candles) we obtained a quotient for $-\frac{\Delta O_2}{\Delta CO_2}$ of $1.08 \pm .03$ which agrees with most of the values for unicellular green algae (Rabinowitch, 1945, p. 33).

It was of interest to see if these blue-green algae could be adapted to utilize hydrogen for reduction of CO_2 in the light, as they had been found in a habitat favoring development of anaerobic organisms. All experiments were successful in which we attempted to adapt these organisms to such a metabolism. Comparable experiments with *Scenedesmus obliquus* (strain D–3) showed that it took longer for equally dense suspensions of the blue-green algae to become adapted than for the green alga *Scenedesmus*. However, once photoreduction had started it would continue for several days at low light intensities.

We have attempted to measure the $H_2 : CO_2$ quotient by a two vessel method using dilute bicarbonate and a carbonate-bicarbonate buffer. Later experiments, with the species of *Chroococcus* mentioned previously, have indicated that the rate of adaptation and of photoreduction is affected by the pH of the medium and thus we believe that we have obtained only an approximate measure of the $H_2 : CO_2$ quotient in this alga.

It is evident from Table I (carbonate-bicarbonate vessel) that the rate of hydrogen absorption increases with increasing light intensity until reversion to normal oxygen evolution occurs. The rate in the light is considerably higher than the rate of gas absorption in the dark, and thus demonstrates the existence of photoreduction in these algae. In these experiments we have no definite proof that CO_2 is reduced simultaneously with the absorption of H_2, in view of the pH sensitivity of the organisms. Gas analyses, however, in experiments with fully adapted *Chroococcus* suspensions have established a $H_2 : CO_2$ quotient in this alga which is close to 2. Only further studies will show if the observed changes in the quotient are significant and to which extent its actual value depends on the state of adaptation of the cells. It is logical that the quotient should change upon de-adaptation of the hydrogenase and should depend upon the state of reduction of the cells.

Adapted cell suspensions of *Synechococcus* and also of *Chroococcus* sp. differ in an interesting manner from those of *Scenedesmus* D–3. De-adaptation of the hydrogenase by increasing the light intensity is a gradual process in these blue-green algae (Table I), and re-adaptation can be brought about at low light intensities. In *Scenedesmus*, reversion to photosynthesis at higher light intensities is more sudden and re-adaptation requires incubation of the suspension in the dark.

Summary

1. Enrichment cultures of *Synechoccus elongatus* Näg., a unicellular blue-green alga, carry out photosynthesis in a carbon-dioxide-bicarbonate buffer with a quotient of $1.08 \pm .03$.

2. This alga has a hydrogenase which can be activated anaerobically in the presence of molecular hydrogen. In the light, adapted algae can carry out photoreduction.

3. In contrast to *Scenedesmus*, activation of the hydrogenase can be carried out at low light intensities.

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PHOTOREACTIVATION OF ULTRAVIOLET LIGHT INJURY IN GAMETES OF THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS [1]

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The finding that visible light can reverse the deleterious effects of ultraviolet radiations (Kelner, 1949a; Prat, 1936; Whitaker, 1942) has resulted in numerous studies attempting to determine how widespread the phenomenon is and the possible mechanism of action of the ultraviolet and the visible light. The majority of these studies have been concerned with reactivation of ultraviolet killed organisms. Photoreactivation of the killing effect of ultraviolet light has been demonstrated on the bacterium, *Escherichia coli* (Kelner, 1949b; Novick and Szilard, 1949) and on the fungi, *Streptomyces griseus* (Kelner, 1949a), *Penicillium notatum*, *Saccharomyces cerevisiae* (Kelner, 1949b), *Ustilago maydis* (Snyder, unpublished), and others. The phenomenon appears to be basically similar in all these organisms. Exposure of ultraviolet inactivated cells to visible light results in a partial recovery of the lost viability.

There are also reports on the photoreactivation of injurious effects other than killing. Reactivation of ultraviolet induced delay in rhizoid formation in zygotes of the alga *Fucus furcatus* (Whitaker, 1942), photoreactivation of ultraviolet retarded adaptive galactozymase formation in *Saccharomyces cerevisiae* (Swenson and Giese, 1950), and photoreactivation of mutations in *E. coli* to bacteriophage resistance (Novick and Szilard, 1949; Kelner, 1949b) serve as examples. Here too, exposure to visible light after or during ultraviolet irradiation causes partial recovery from the ultraviolet injury.

The experimental conditions necessary to demonstrate photoreactivation in ultraviolet inactivated bacteriophage differ somewhat from the above. The inactivated phage particles alone can not be photoreactivated by visible light, but must be adsorbed onto bacteria before the phenomenon will occur (Dulbecco, 1950).

Blum and his co-workers reported photoreactivation in eggs of the sea urchin *Arbacia punctulata* (Blum, Robinson and Loos, 1949; Blum, Loos, Price and Robinson, 1949). Marshak studied photoreactivation in gametes and zygotes of this sea urchin (Marshak, 1949). He finds no photoreactivation of ultraviolet injury to either gamete alone, but that this does occur if the visible light follows fertilization. He analogizes this situation to that in bacteriophage.

The present study is concerned with photoreactivation in gametes and zygotes of the sea urchin, *Strongylocentrotus purpuratus*. In the literature on photoreactivation, work with monochromatic ultraviolet light is strikingly absent, yet the effects of various wavelengths may be quite different. Therefore, visible light reversal of injury caused to eggs by different monochromatic wavelengths of ultraviolet light

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has been attempted and the region of effective visible light defined. Effects of different dosages of ultraviolet radiations have also been studied. Photoreactivation has been attempted before and after fertilization of the injured gametes, and the effects of visible light on sperm investigated.

Materials and Methods

Sea urchins were collected at Moss Beach, California and later in the breeding season at the mouth of Malpaso Creek near Point Lobos, California, and brought to Stanford University where they were kept at 5° C. for two or three days during which time the gametes were used for experiments. The animals from the Malpaso Creek collecting station, which show a delayed breeding season, were often kept for several days in the aquaria of the Hopkins Marine Station at Pacific Grove, California before transport to Stanford University. There was little deterioration of gametes under these conditions of storage, and no detectable difference between material from the two collecting stations.

Eggs were obtained by cutting open a sea urchin to expose the gonads, rinsing with sea water, and taking some of the eggs which spew out when an ovary is slightly injured. Care was taken to avoid contact of the eggs with body fluids or any organs of the animal. The entire testes were cut out of the males and sperms were taken from these when required. Sperms were used in dilution of 1/400, or else insemination was accomplished by simply touching the tip of a dissecting needle to the testis, and then running this through the egg dish. Only gametes which showed almost 100 per cent fertilization and good early development were used for experiments. Sea water was obtained at the same places as were the urchins. It was filtered immediately and pH determinations showed that it did not change much on standing thereafter.

Dishes were made from the bottoms of new glass vials and were carefully tested to show that they supported normal development before being used in experiments. All operations except the irradiations with the monochromator were performed in a 13° C. constant temperature room. Monochromator irradiations were done at room temperature. Tests showed that such brief exposures to room temperature before fertilization had no detectable effect on the gametes.

The ultraviolet irradiation technique was the same as previously described (Giese, 1938a, b) and the eggs were handled in essentially the same manner. In essence, the source of radiations was a quartz mercury arc used with a natural quartz monochromator. The intensity of a desired wavelength of light was measured at the time of the experiment with a thermopile calibrated against a Bureau of Standards Standard Lamp.

After ultraviolet irradiation, the gametes were carefully screened from visible light, except for controlled exposure during photoreactivation. For this the brilliant light of a 100 Watt G. E. projection spotlamp was used. The lamp was 70 cm. from the eggs and the light passed through 20 cm. of water to remove heat rays and a Corning No. 3060 glass filter to remove short ultraviolet. This filter has a transmission from about λ3700Å through the visible (50 per cent transmission at λ4100Å), and allows good photoreactivation. Blue light from the monochromator (λ4350Å) was used in work with sperm.

Delay in cleavage of the zygotes (formed from union of ultraviolet irradiated gametes of one sex with normal gametes of the opposite sex) was taken as the criterion of injury, and reduction of this ultraviolet induced delay by visible light as photoreactivation. The time at which 50 per cent of an experimental sample of zygotes were divided was taken as the point of cleavage. The method routinely used for obtaining data on the per cent cleavage in a sample was to photograph it at intervals during the course of the experiment in essentially the same way as was done by Blum and Price (1950a, b). This was done with a Leica camera through a Microibso attachment, using an ordinary Leitz monocular microscope with a Leitz No. 2 objective. This gives a field large enough to include one or two hun-

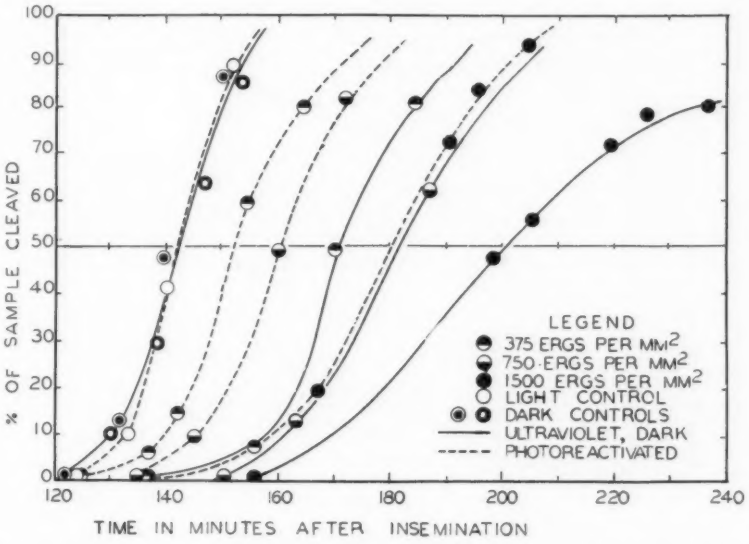

FIGURE 1. Delay in cleavage of sea urchin eggs injured with various dosages of ultraviolet wavelength 2450Å, with and without photoreactivation. All irradiations completed before fertilization. Visible light exposures are the same in each case. Dosage series at λ2537Å and λ2654Å show similar cleavage characteristics.

dred eggs in each photograph. The microscope light was filtered through a Corning No. 3480 Glass filter which transmits only to λ5500Å in the visible (50 per cent transmission at λ5700Å). The filter effectively eliminated photoreactivation from this source and a 5 cm. water cell removed any heat. Exposures of two and one-half seconds on Kodak Microfile film were adequate under the lighting conditions used. The negatives obtained in this way were projected with an enlarger and counts made from them. This method gave a permanent record which could be read at leisure. For critical experiments, the counts were made a number of times and their results were found to be quite repeatable. Experiments were usually repeated three times, and dark and light controls were run on each experiment.

Experimental

In the first group of experiments, the effects of series of dosages of ultraviolet light at λ2450Å, λ2537Å, and λ2654Å were determined. Eggs were irradiated with the indicated dosages of ultraviolet, then exposed to white light from the G. E. spot-lamp for one-half hour, and fertilized with normal sperm. Each series was done with eggs from a single female and sperm from a single male. Data on cleavage were determined as described above, plotted, and the points of 50 per cent cleavage determined. Figure 1 shows the cleavage characteristics of such a series at λ2450Å. The points of 50 per cent cleavage from Figure 1 and from similar series of dosages at λ2537Å and λ2654Å are plotted in Figure 2. In each of these figures, solid lines

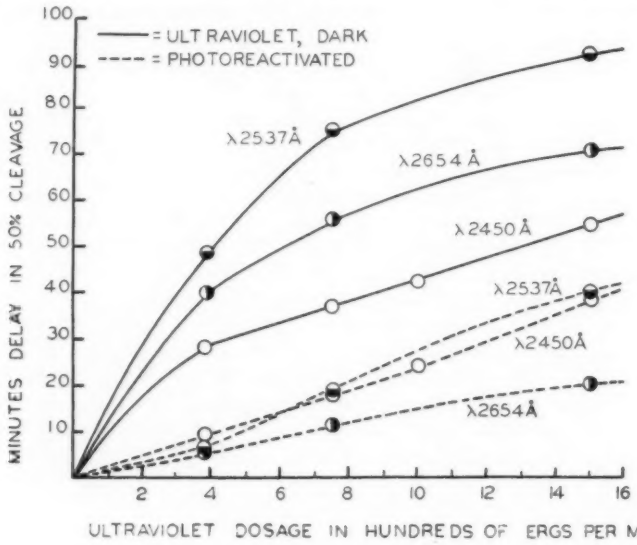

Figure 2. Photoreactivation of injury to eggs caused by wavelengths 2450Å, 2537Å, and 2654Å. Times of 50 per cent cleavage of the samples of eggs are compared here. Visible light dosages were the same in each experiment. All irradiations were completed before insemination.

represent dark controls and ultraviolet injured eggs. The dotted lines represent visible light controls and photoreactivated samples.

Complete dosage series were not run on other wavelengths, but representative dosages were tested. In Table I some of these data are compared, data from Figure 2 being included. Visible light exposure was held constant for all of these experiments.

Photoreactivation occurred for all wavelengths tested and for each trial at any wavelength. Comparison of efficiencies of the process at different wavelengths is difficult because the wavelengths of ultraviolet light vary in the amount of injury caused by a given exposure. The action spectra for ultraviolet light injury to sea urchin gametes have been previously described (Giese, 1946). There is also varia-

TABLE I

Representative photoreactivation data from various wavelengths of ultraviolet light, keeping the visible light dosage constant. Controls are taken as zero delay.

Wavelength of ultraviolet, in Angstrom units	Dosage of ultraviolet, in ergs/mm.²	Cleavage delay, in minutes; ultraviolet	Cleavage delay, in minutes; photoreactivated	Per cent photoreactivation
Control	0	0	0	0
2450	750	37	18	51
2450	1500	55	18	31
2537	750	76	19	75
2537	750	36	6	83
2537	1500	93	40	57
2654	750	56	11	80.5
2654	750	51	14	72.5
2654	1500	71	20	71.7
2804	750	90	26	71
3025	750	37	11	70
3130	6000	14	2	86

tion in the sensitivity to ultraviolet injury in eggs from different females. It seems, however, from the data available (Figure 2 and Table I), that there is strikingly less reactivation from λ2450Å injury than from injury by other wavelengths. Furthermore, there is a marked unilateral effect in raising of the membrane at

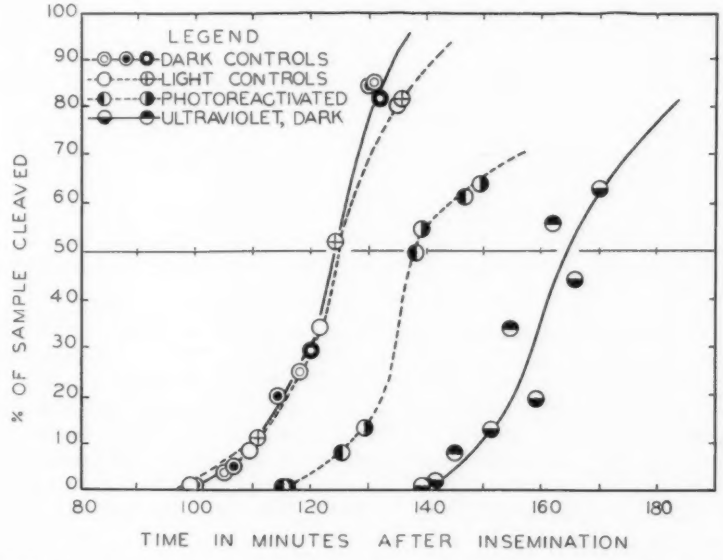

FIGURE 3. Photoreactivation of zygotes formed from eggs injured by 750 ergs/mm.² of λ2654Å ultraviolet light. Visible light exposure of ⅛, used in the experiments of Figure 1 and Figure 2, was immediately after fertilization. Photoreactivation of 65.5 per cent was obtained.

λ2450Å which is not usually apparent at other wavelengths. This suggests that injury from superficially absorbed ultraviolet light is not so readily photoreactivated as injury caused by wavelengths which are more strongly absorbed by nucleoproteins. More data are necessary for a complete analysis of this situation.

In all of the experiments discussed above, eggs were photoreactivated with one-half hour exposures under the G. E. spotlamp, then fertilized with normal sperm. This was found by experimentation to be the minimal dosage which would give an effect reasonably near maximal. If a Corning No. 3389 glass filter was substituted for the Corning No. 3060 filter routinely used on the reactivating lamp, photoreactivation was greatly reduced. The Corning No. 3389 filter transmits only wavelengths longer than λ4100Å (50 per cent transmission at λ4300Å). It has been

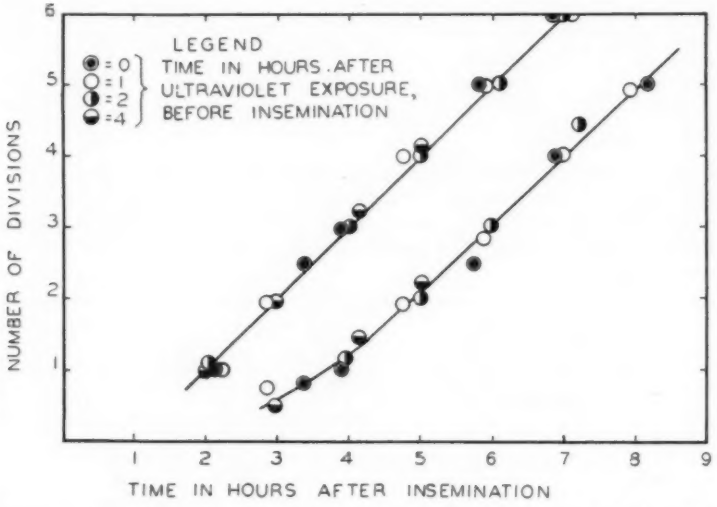

FIGURE 4. Comparison of cleavage rates of sea urchin eggs injured by ultraviolet light, then kept in the dark for various periods of time before insemination. Two experiments are shown.

shown on other organisms that the spectral region λ3660Å to λ4300Å is usually most effective in photoreactivation (Dulbecco, 1950; Kelner, 1950). Our experiments show that in Strongylocentrotus, wavelengths shorter than λ4300Å are most effective.

That fertilization prior to photoreactivation is not required is indicated by all of the experiments thus far described. It was found, however, that after fertilization of injured gametes, photoreactivation of the resulting zygotes was somewhat more easily accomplished. Visible light dosages of ten minutes sufficed to cause reactivation of the same magnitude as was caused by thirty minutes exposure on the unfertilized eggs. Figure 3 shows the results of one experiment on reactivation of zygotes.

Eggs of Strongylocentrotus do not show any measurable increase or decrease in amount of delay caused by an ultraviolet exposure, when they are kept in the dark

in sea water. This was experimentally shown by irradiating samples of eggs with ultraviolet light from a sterilamp (arbitrary exposure of sixteen seconds), and fertilizing portions of the samples at intervals after the irradiation. Samples were followed through the sixth division in order to detect any change in rate of cleavage caused by the delay in fertilization. Portions of each sample were inseminated immediately after irradiation, and at one, two, and four hours thereafter. Figure 4 shows the results of two such experiments. Note that the delay of cleavage for all sample portions of irradiated eggs is practically identical.

Sea urchin sperm are much more sensitive to ultraviolet light injury than the eggs. Fifty ergs of λ2654Å suffices to cause a delay in cleavage of 80 to 100 minutes

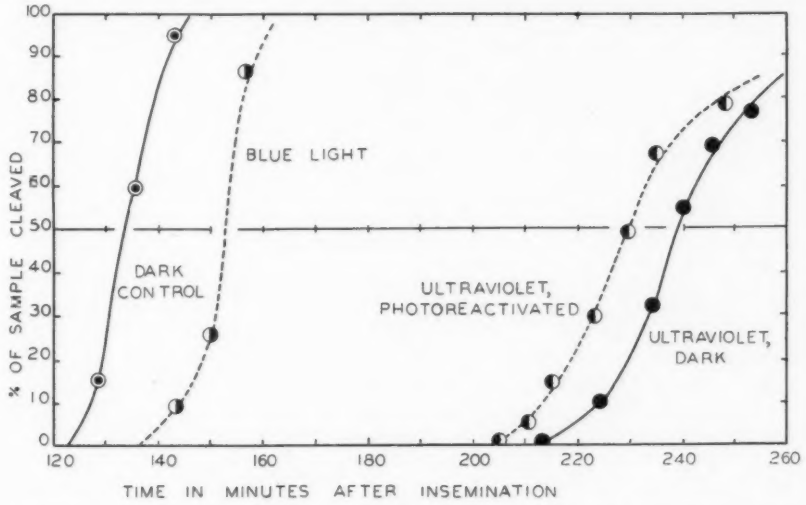

FIGURE 5. Photoreactivation of ultraviolet injured sperm. Sperm were injured with 50 ergs/mm.^{2} of λ2654Å ultraviolet, photoreactivated with 4086 ergs/mm.^{2} of λ4350Å blue light, then used to fertilize normal eggs. Cleavage delay in the resulting zygotes is shown. Note the retardation of cleavage in the blue light control.

in zygotes from such injured sperm. An action spectrum has been worked out which shows that this wavelength is near the peak of efficiency for ultraviolet injury to sperm, and a detailed comparison of ultraviolet effects on eggs and sperm has previously been made (Giese, 1946, 1949). The most obvious effect of ultraviolet light injury to sperm is retardation in the cleavage of zygotes resulting when injured sperm are used to fertilize eggs. Even after quite large dosages of ultraviolet, the sperm remain motile and activate the eggs.

Visible light is also very harmful to sperm. Its effects will be reported in detail elsewhere, but it is necessary to present some of the data here, in connection with photoreactivation in sperm. The visible light injury is qualitatively different from ultraviolet light injury. Sperm exposed to visible light become immotile and fail

to activate the eggs. Sensitivity varies with gametes from different individuals. Usually the dosage which will kill nearly 100 per cent of the sperm is between 20,000 ergs/mm.^{2} and 40,000 ergs/mm.^{2} for monochromatic blue light of λ4350Å (the wavelength used in photoreactivation experiments with sperm). Sublethal dosages cause a delay in cleavage of the zygotes formed from sperm so injured. This delay is of a lower order of magnitude, however, than the ultraviolet induced delay.

The injury caused by ultraviolet light on sperm appears to be different from the injury caused by visible light. Visible light of low intensity might, therefore, cause photoreactivation of ultraviolet induced delay in cleavage, even though the visible light is itself injurious.

In order to test this, sperm suspensions of 1/400 were irradiated in a quartz vessel in the monochromator with 50 ergs/mm.^{2} of λ2654Å ultraviolet, followed by sublethal dosages of blue λ4350Å (4000 to 5000 ergs/mm.^{2}), appropriate controls being run. Normal eggs were then fertilized with these sperm, and the time of cleavage determined. Photoreactivation was in fact demonstrated. Figure 5 shows one such experiment. This was repeated three times with similar results. Note the retardation of cleavage in the blue light control. In spite of this injury, and in spite of the relatively low dosage of visible light used, a photoreactivation of ten minutes is observed.

Discussion

Most previous studies on photoreactivation have been with a sterilamp as the source of ultraviolet radiations. Such sterilamps give off mixed wavelengths of ultraviolet with maximum intensity at λ2537Å. The data presented here indicate that cleavage delay caused by any of the wavelengths of ultraviolet tried is photoreversable, but that λ2450Å injury is less so than injury by wavelengths longer than this. λ2450Å is strongly absorbed by surface proteins, as indicated by the unilateral effect obtained in raising of the membranes when eggs are injured by this wavelength. Several wavelengths tested are much more selectively absorbed by nucleoproteins. It is suggested, then, that injury to nucleoproteins is most strongly photoreactivated. This should be more adequately investigated. It is of interest to note that wavelengths λ2654Å and longer do not produce appreciable amounts of ozone. Therefore, the reversable injury is not due to ozone formed by the ultraviolet light.

Marshak (1949) has found that in Arbacia the injured gametes cannot be photoreactivated, but that zygotes formed from injured gametes are quite sensitive. The data on Strongylocentrotus do not agree with these findings. Either there is a great difference in these two sea urchins with respect to photoreactivation, or the dissimilarities must be accounted for by differences in technique. Since the injured eggs in Strongylocentrotus are less sensitive than zygotes formed from them, perhaps the visible light illumination periods used on Arbacia eggs were too short to cause measurable photoreactivation. Failure of ultraviolet injured sperm to be photoreactivated may have been due to visible light injury to them.

It has been suggested (Novick and Szilard, 1949) that the injurious effect of ultraviolet light may be due to the formation of some sort of poison. Since photo-

reactivation is never complete and is possible only for a time after ultraviolet irradiation, they suggest that the poison has two forms, one stable to visible light and one photolabile. As time passes after injury, the second is largely transformed to the first. Hertel (1905) discussed the possibility of the ultraviolet effect being due to the formation of a poison. He found that if one of a pair of blastomeres was irradiated, the second would be effected, provided the cleavage plane had not yet entirely separated the two. The second blastomere, he argued, was affected by some toxic substance (poison) produced in the first and diffusing into the second.

If poison production is, in fact, the mechanism by which cells are injured by ultraviolet light, this poison must be of a size or form which prevents diffusion through the cell membrane. Hertel's experiment works only if the cleavage plane does not completely separate the blastomeres. Photolyzed eggs added to normal ones do not in any measurable way affect their cleavage. Ultraviolet light injured eggs of Strongylocentrotus do not show recovery, even after standing for hours in sea water. If the injurious substance were able to diffuse into or out of the cell through the membrane, it should certainly be demonstrable by these experiments.

Synthetic processes of the cell seem to be quite sensitive to ultraviolet radiation injury (Giese, 1950; Swenson and Giese, 1950). It can be hypothesized that the photoreversable effects of these radiations, such as killing, inhibition of adaptive enzyme formation, and cleavage delay, are all manifestations of a basic disruption of the synthetic processes of the cell. Failure to obtain complete photoreactivation suggests a multiple effect of ultraviolet light.

Summary

1. Ultraviolet radiation induced cleavage delay in eggs of the sea urchin *Strongylocentrotus purpuratus* can be reduced by exposure to visible light before fertilization.

2. Photoreactivation by visible light occurs in injury by any of the wavelengths of ultraviolet light which we tested: λ2450Å, λ2537Å, λ2654Å, λ2804Å, λ3025Å, λ3130Å. The phenomenon is less pronounced at λ2450Å than at the other wavelenths tried.

3. Wavelengths shorter than λ4300Å are most effective in photoreactivation. The minimum visible light exposure giving an effect near maximal is determined.

4. Zygotes formed from ultraviolet injured eggs are more readily photoreactivated than the unfertilized eggs.

5. Ultraviolet irradiated eggs show no increase or decrease in injury when kept in the dark for several hours before fertilization.

6. Visible light is injurious to sperm.

7. Sperm injured by ultraviolet radiations can be photoreactivated, even though the visible light is itself harmful.

8. The results are compared with photoreactivation data in the literature and discussed with reference to possible mechanisms of action for the phenomena observed.

We are indebted to Dr. L. R. Blinks and Dr. R. L. Bolin for many courtesies extended to us at the Hopkins Marine Station, and especially to Dr. C. S. Yocum for help in obtaining suitable biological material.

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CYST-FORMATION IN AEOLOSOMA HEMPRICHI (EHR)

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Cyst-formation has not often been observed in the Oligochaeta. It was observed for the first time by Beddard (1905a, 1905b) for the species *Aeolosoma hemprichi* Ehr. It was his opinion that cyst-formation appearing at the beginning of winter is due to the decrease of the temperature. According to Vejdowsky (1884), this phenomenon is a resting stage following a long period of asexual reproduction; while Dehorne (1916) considers it to be consecutive to sexual reproduction, which effectively occurs in the months of November and December, Beddard (1889) and Stolc (1889).

The opportunity to study this phenomenon occurred during my sojourn in Canada, at the University of Montreal. The author wishes to express her indebtedness to the Board of Trustees of this Institution; to Dr. Henri Prat, Director of the Institute of Biology and to Father O. Fournier, Assistant-Director, who helped us greatly in the accomplishment of this work. I wish to acknowledge the active collaboration of Miss Marthe Demers.

Scope of the Work

Our concern is cyst-formation in *Aelosoma hemprichi* Ehr, induced experimentally in order to follow the successive stages from the beginning of the phenomenon until the emergence of the encysted worm. These observations also permit a study of the causes that determine cyst-formation in natural surroundings, and the related factors and the importance of the phenomenon in the biological cycle of the worm.

Materials and Technique

The genus Aeolosoma

While studying the fresh-water fauna of some brooks in the region of Montreal, the three following species of Aeolosomatidae were encountered: *A. hemprichi*, *A. haedleyi* and *A. tenebrarum*. Their systematic position will be studied in another report. This paper will be restricted to the species *A. hemprichi*. The genus Aeolosoma is easily recognized by the ciliated prostomium and the lipoidic inclusions in the skin. The color of these inclusions varies with the species: in *Aeolosoma hemprichi* they are red. The presence of setae and of nephridia is the only means of recognizing metamerisation, since Aeolosomatidae possess neither intersegmentary septa, nor nerve cord with segmentary ganglia.

The transparency of the worm facilitates the study of the internal organs, especially the components of the digestive tract: the ciliated prostomium, the muscular pharynx, the narrow esophagus and the large stomach which gradually merges with the intestine.

This worm reproduces asexually very rapidly. The laws governing this scissiparity have been studied previously (Meewis, 1933). They may be summarized as follows:

(1). Every individual is composed of six seta-bearing segments, the pharyngeal, the esophageal and the four stomacho-intestinal segments. As soon as these have appeared, the pygidium becomes longer and divides into two parts: a small anterior one which will regenerate the pygidium and a longer posterior one which becomes a new zoid. This mode of scissiparity was previously termed "pygidial budding."

(2). When the pygidial bud is formed, it has no seta. However, the first groups of setae soon appear, and progressively, as the bud grows longer, the following groups are formed. When the bud reaches the five seta-bearing segment, all the setae are practically equidistant. Simultaneously, the cephalic zone develops and the pharyngeal setae appear at the anterior part of the bud. These are at first close to the esophageal setae, but as the esophagus grows longer they separate from each other. The zooid is then complete, and possesses a head, six seta-bearing segments and a pygidium capable of budding.

(3). The initial bud composed of six segments will now present a secondary bud.

(4). When cephalization is complete, the initial bud separates from the chain bearing the buds to which it gave rise. Thus, true scissiparity which divides an individual into two parts, more or less equal, and which characterizes fresh-water Oligochaeta, does not exist in this species. By means of an accelerated process, blastogenesis takes place exclusively by pygidial budding.

Aeolosoma hemprichi, represented in Plate I, Figure 1, is composed of an initial individual followed by the buds formed by its pygidium. It corresponds to the formula 6/0/3/6/0, which represents the number of seta-bearing segments of each successive zooid.

This pygidial budding forms chains comprising from two to six individuals or zooids, and of a mean length of 2 mm.

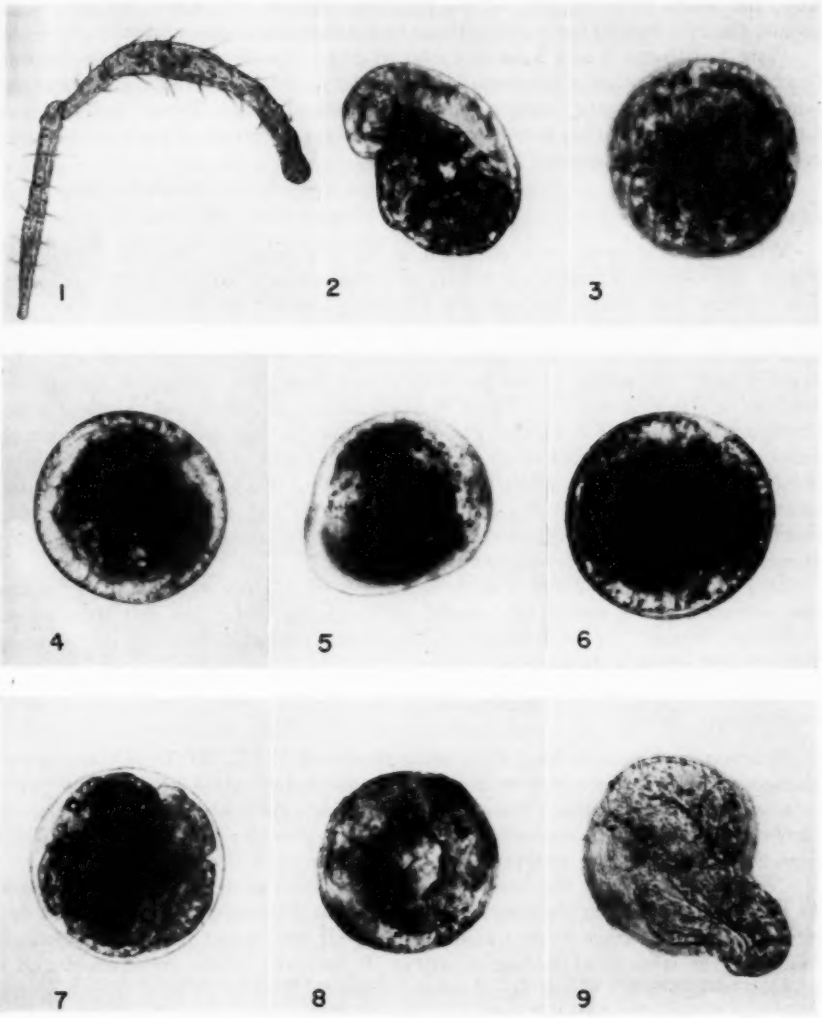
When environmental conditions are stable, pygidial budding occurs regularly and rapidly. The rate of growth varies with the amount of food available and the temperature.

In order to study the influence of environmental conditions on the laws of scissiparity, some cultures were kept at different temperatures ranging from 11° C. to 29° C., whereas the stock culture was kept in front of the laboratory window. Because of a sudden cold spell in the middle of the winter this stock culture was submitted to a temperature of 6° C.–7° C. for about 10 days and it was at this time that the first cyst-formation was noticed.

Description of the cyst

The cysts are generally spherical and of variable size, measuring from 175 to 250 microns in diameter; some may have irregular forms. The worm is easily visible through the thin and completely transparent membrane. The skin of the worm is in close contact with the cyst-membrane, the latter appearing as though spotted with red. The digestive tract, which is very opaque, is joined to the skin by means of mesenteric filaments.

Within the cyst, in cold surroundings, the worm is motionless and its contours difficult to see, though the peristaltic movements of the intestine make orientation



EXPLANATION OF PLATE I

1. *Acolosoma hemprichi:* Photograph of a live normal worm; 2. Pre-cystic stage; 3. Beginning of cyst-formation; 4. Quiescent cyst: equatorial view; 5. Quiescent cyst: tangential view; 6. Beginning of activation; 7. Activating cyst; 8. Pre-emergence stage; and 9. Emergence stage.

possible. When seen through the microscope, because of the heat emitted by the lamp, the worm turns around within the cyst. When this occurs its skin slides against the cyst membrane making these two tissues distinctly visible.

Plate I, Figures 4 and 5 show a cyst at 5° C.; the first one being a medium (equatorial); the other a tangential view (polar), shows the red pigments of the skin, the cyst membrane being quite transparent. Plate I, Figure 6 shows that the space between the cyst membrane and the worm is increased by its movements, as a result of the heat emitted by the lamp.

Cyst formation

When the first cysts were observed at the beginning of December, 1949, the culture still contained free living worms on which we were able to study cyst formation. These free worms present the following aspect: the chains are short and composed of two zooids corresponding at the most to the formula 6/1, *i.e.*, a complete anterior zooid of six seta-bearing segments, followed by a small non-segmented pygidial bud. According to our previous observations, this formula is the simplest one applying to this species. These individuals become bulky and opaque because of the accumulation of refractive granules around the digestive tract. Simultaneously, their movements slow down; whereas normal worms, because of their ciliated prostomium, move about rapidly in a way resembling that of rhabdocoela. Worms submitted to low temperatures crawl, change form, and evidently prefer regions rich in organic debris. While crawling and turning about they secrete a heavy mucus which hardens to form the cyst membrane.

In Plate I, Figures 2 and 3 show two stages of this phenomenon. In the first one, secretion of the mucus has started, although the worm can still stretch itself and move around. In the second case, the worm is imprisoned in its mucus which hardens and forms a shell-like covering.

Activation of the cyst

When a cyst is submitted to a temperature of 18° C.–20° C., its appearance changes rapidly. The worm turns around continuously while its head and pygidium, which were indistinct, become visible again. At the same time, the worm recovers its initial transparency; the refractive granules which surrounded the digestive tract disappear progressively.

Nevertheless, during the first few days, the worm (as shown on Plate I, Figure 6) is still as bulky as at the time of the formation of the cyst. After 8 to 10 days it reaches another stage in its transformation. It grows and bears new segments, following the laws of normal scissiparity. It becomes totally transparent and in every way resembles a free-living worm. Simultaneously its movements become very rapid, permitting it to turn over very quickly within its cyst. The growth can be followed by the study of the seta and this is rendered possible by the transparency of the cyst membrane. After twelve days, generally, at this temperature (18° C.–20° C.) the worm endeavors to free itself. In certain cases, emergence from the cyst seems easy enough: the membrane is first slightly ruptured, the worm then pushes its pygidium out through this opening and the remainder of its body follows. This phenomenon can take place within half an hour or sometimes necessi-

tates several hours. It may happen that the cyst is covered with foreign matter or with a heavy coating of bacteria. When this occurs the worm cannot easily break through its cyst membrane and it may remain imprisoned for several days. In this case, its growth progresses as if it were free, producing new zooids, and thus there may be several chains within the cyst. This would explain the exceptional finding of three worms within a single cyst. But when the worm emerges under normal conditions it is generally composed of two zooids answering to the formula 6/3, *i.e.*, the chain is composed of a complete anterior individual with six seta-bearing segments and a three-segment pygidial bud.

It does not seem that emergence is caused by the action of a corrosive substance secreted by the worm, but is purely mechanical.

As soon as the worm is free it grows normally, its growth being regulated by the environmental temperature and the quality of the food present. It may live normally without eating for several days, but if the fasting persists, once the reserve substances have been used up, the worm grows thin and its coloring disappears. The effect of fasting on these animals has been previously described.

Plate I, Figures 7, 8, and 9 show three successive stages under heat activation. The worm appearing in Figure 5 is still bulky but its outline is quite distinct. In Figure 8 the worm has reached the pre-emergence stage and in Figure 9 it is emerging. We can observe, in the emerged part, the limit existing between the two zooids which make up the chain.

We can summarize the successive stages of cyst formation and emergence in *Aeolosoma hemprichi* as follows:

(1). Normal worm: (N.W.) the chains are composed of 2 to 6 individuals, the animal is transparent and moves rapidly.

(2). Depressed worm: (D.W.) the chains are of normal length but the movements are slower.

(3). Pre-cystic stage: (P.C.) the chains are made up of 2 individuals, at the most. The worm is bulky and opaque, its movements are very slow.

(4). Cyst-formation: (C.F.) the worm turns around in the secreting cyst.

(5). Quiescent cyst: (C.) the worm is motionless and its contours are indistinct.

(6). Activating cyst: (A.C.) the worm moves around and becomes transparent.

(7). Pre-emergence stage: (P.E.) the very active worm lengthens and tries to rupture the cyst.

(8). Emergence: (E.) the worm ruptures the cyst.

Experimental Study of the Factors Determining Cyst-Formation

Effect of low temperatures

No cyst is formed in cultures which are not subjected to low temperature from June to December: the worms undergo asexual reproduction.

Three groups of two hundred and fifty worms each were taken from a culture maintained at 18° C. and placed in an infusion rich in microorganisms. These cultures were then kept at three temperatures: 3° C., 6° C. and 11° C. The results are summarized in Table I.

These results show that:

1. At 3° C., the movements of normal worms are retarded and only a certain number of them attain the cystic stage. But these individuals seem less bulky and

less opaque than individuals at normal pre-cystic stages. The majority of them resemble fasting worms, as described earlier.

2. At 6° C., slowing of movements is followed by the formation of a normal pre-cystic stage. The first cysts appear after the worms have been kept about 15 days at this temperature. About 50 per cent of them, however, do not go through cyst-formation and remain in a pre-cystic stage for two months.

TABLE I

Duration: days	0	3	7	15	30	60
Exp. at 3° C.	N.W.	D.W.	A few P.C.	A few P.C.	A few P.C.	A few P.C.
Exp. at 6° C.	N.W.	D.W.	P.C.	First C.	Many C.; remainder P.C.	Many C.; remainder P.C.
Exp. at 11° C.	N.W.	D.W.	D.W.	D.W.	D.W.	D.W.

N.W.—normal worm; D.W.—depressed worm; P.C.—pre-cystic stage; C.—cyst.

3. At 11° C., activity is reduced and asexual reproduction is slower; the worms do not reach the pre-cystic stage, the long chains persist and the growth is similar to the one observed during summer months at the same temperature.

We can conclude as follows: (1) The optimum temperature for cyst-formation appears to be about 6° C. (2) The cysts are formed after about 15 days at a low temperature, but this value differs with each individual of the culture. (3) A low temperature is not alone responsible for cyst-formation, since this phenomenon takes place in part of the population at 6° C., and does not affect worms kept at 3° C.

Effect of food

To study those conditions besides temperature which must operate in order to produce cyst-formation, some worms were taken from a culture kept at 18° C. and rich in nutritive substances, and were placed in pure water at 3° C. and 6° C. At both temperatures the movements of the animals were slowed down but pre-cystic stages did not appear; after fifteen days the worms were either dying or dead.

Effect of the combined factors of temperature and food

Different combinations of these two factors were studied so as to determine precisely the role of the nutritive medium during the different stages of cyst-formation.

Two hundred and fifty normal worms were placed at 6° C. in a rich nutritive medium; when they reached the pre-cystic stage, they were divided into two groups: one was kept in the same conditions of temperature and food, while the other was placed in pure water at the same temperature. After seven days, the worms from the first group were encysting while those from the second group were nearly all dead, none having reached the cyst stage. So that besides being necessary for cyst-formation, food is also necessary after the pre-cystic stage.

The final experiments help to explain the results first obtained.

1. At 3° C., the microorganisms on which the worms feed no longer multiply actively and the medium becomes poorer in nutritive substances up to a stage where no cyst-formation can take place. Therefore, if this medium is renewed periodically, to supply sufficient food, normal pre-cystic stages will appear after 15 days and the first cysts within a month. Cyst-formation can take place at 3° C. provided the culture medium is constantly replenished; however, this phenomenon proceeds more slowly than at 6° C.

2. We have seen previously that if cultures which are kept at 6° C. (where cyst-formation is possible) are not maintained at a high nutritive level, a certain number of the worms will remain in a pre-cystic stage; these individuals thrive because enough food is present, but it is impossible for them to transform into cysts. Such pre-cystic worms, taken from a culture where no cyst-formation had resulted after one month at a low temperature, were placed in a rich nutritive medium: three days later, cysts appeared.

The influence of food is therefore obvious; the worm must be able to accumulate reserves during the stages preceding cyst-formation. The elaboration and the nature of these reserves will be studied in a future paper. The worm is especially in need of these reserves during its encysted and emergence stages.

Discussion and Conclusions

The phenomenon of cyst-formation cannot be considered solely as a resting stage occurring after a long period of asexual reproduction as proposed by Vejdowsky, for no cysts were formed in cultures kept in winter at temperatures above 11° C. Neither can it be considered as a quiescent period following sexual reproduction, as thought by Dehorne, for that type of reproduction did not occur at all in our cultures. But cyst-formation is certainly affected by low temperatures as stated by Beddard, and this phenomenon takes place only when sufficient food is present so that reserves can be accumulated.

There exist several groups of fresh-water animals which hibernate during the course of their biological cycles; for example, the Bryozoa survive the winter period as statoblasts or hibernacula (Brien, 1936) and the sponges of the Spongillidae family, as gemmules (Brien, 1932; Leveaux, 1939). In both instances, the organisms could not otherwise withstand the winter cold. They form buds which consist of a mass of undifferentiated cells containing large quantities of nutritive reserves and surrounded by a protective covering. In the following spring, these cells multiply and differentiate, the shell splits open and a new individual escapes.

The same cannot be said of Aeolosoma. Here, the whole animal is enclosed within the cyst and its life processes are slowed down. Transformations occurring in the reserve tissues are not as pronounced as in Bryozoa or sponges.

In natural surroundings, cyst-formation may be explained in the following manner: in autumn, the water becomes colder and richer in products of decomposition from vegetable matter: Aeolosoma then stores its reserves. When the water is sufficiently cold, the worm becomes encysted and falls to the bottom of the creek into some hollow spot which does not freeze. In this way the worm can resist a temperature of a few degrees above 0° C. during winter. When the water warms up in spring, the worm emerges and asexual reproduction is resumed. The biological cycle can thus take place without the occurrence of sexual reproduction.

However, this last type of reproduction was observed in a few cases in *Aeolosoma hemprichi* and *Aeolosoma quaternarum* but it did not occur in any of our cultures though submitted to various environmental conditions. Here, as in other limicolous Oligochaeta, for example *Chaetogaster diaphanus*, where asexual reproduction is very intense, sexuality is a secondary phenomenon which appears only in certain definite circumstances. Scissiparity alone can definitively assure the survival of the species, and in places where the winter months are rather cold, the individuals survive by encystment.

Summary

(1). The fresh-water oligochaete, *Aeolosoma hemprichi*, hibernates in the form of a cyst.

(2). When the worms are placed in a rich nutritive medium at 6° C., their movements are slowed down, and their growth stops. At this period, chains are formed of two zooids, at the most. Meanwhile, the worm accumulates food reserves, becomes opaque and secretes a mucus which will harden and form a transparent cyst.

(3). The worm stays motionless inside the cyst during the entire cold period. When the temperature increases, it becomes active again, uses up its food reserve and becomes transparent. After about fifteen days, it emerges and resumes its growth according to certain definite laws of scissiparity particular to this species.

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THE RELATION BETWEEN THE SCATTERING LAYER AND THE EUPHAUSIACEA[1]

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Introduction

During and since the war, workers in various laboratories have reported that in the process of underwater sound experiments, mid-water echoes have been obtained from what has come to be referred to as the "Deep Scattering Layer." Results of observations made by the University of California have appeared in a series of reports (Univ. Cal., 1942–1946), while others from the Woods Hole Oceanographic Institution have been published by Hersey and Moore (1948), and Moore (1948). Papers by Raitt (1948), Dietz (1948) and Johnson (1948) describe more recent work on the subject. Most of the work which has so far been done on the scattering layer has been of a physical nature, and it seems most desirable at this stage that the problem should be discussed from a biological aspect.

It is a difficult matter to prove directly what material is responsible for an echo in the sea. The alternative approach is to determine what materials present in the sea are capable of producing echoes of the observed type and then to determine which of these exhibit identical characteristics of distribution, diurnal migration, etc., with the echoes. These characteristics are of so complex a type that, if an organism can be found which parallels them, the probability becomes extremely high that it is responsible for the observed echo phenomena. It must be realized that throughout this paper we are endeavoring to make such a comparison: available data on the scattering layer and on certain zooplankton are compared, and at the end an estimate of the probability of the correctness of the solution, rather than a proof, is presented.

This paper is presented in three sections: the first deals mainly with the scattering layer, the second with the biology of euphausids and the appendix with the computation of illumination. Since work on all lines has developed concurrently, it is not possible to separate them completely, and in many cases it is necessary to interpret observations made in one section in terms of observations in the other section.

The Scattering Layers

General. Most of the data discussed here have been obtained from bathygrams.[2] Although oscilloscope records of individual signals afford much greater detail, the continuous spark-recordings have the great advantage of allowing the complete

[1] Contribution No. 535 from Woods Hole Oceanographic Institution. Contribution No. 47 from University of Miami Marine Laboratory.

[2] There appears to be need for a term descriptive of records from echo sounders, and not suggestive, by implication, of a particular trade product. For this reason we propose the word "Bathygram," and use it throughout the paper.

picture for a twenty-four hour period to be examined as a whole. The scattering layer usually appears on these bathygrams as a darkened strip, lying, in the daytime, at a depth of two to three hundred fathoms. Between sunset and sunrise it approaches the surface, and usually is masked there by the record of the outgoing signal (Fig. 1). Most often there is only a single layer, but on a number of occasions two or more distinct layers have been recorded, and there is little doubt that improved techniques, including, especially, shortening of the ping length, will allow the separation of such multiple layers more regularly in the future. There may also be layers which do not execute the same type of diurnal migration as the main layer. Finally there is often a very deep layer lying at a depth of about five hun-

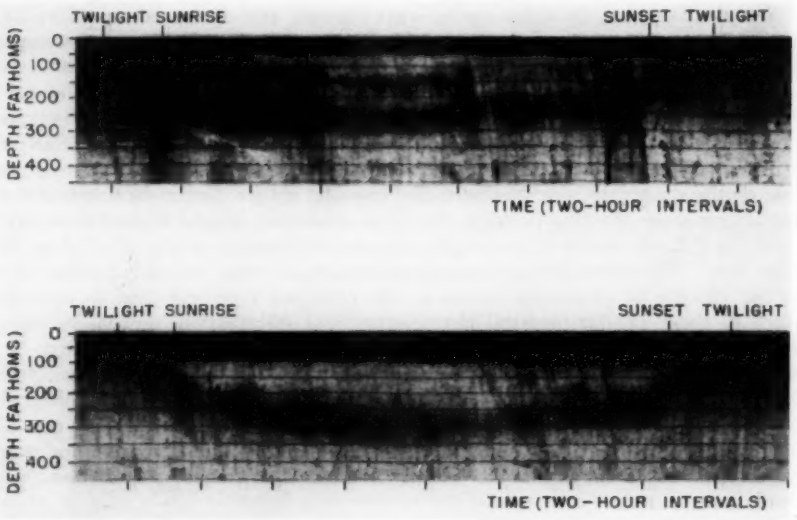

FIGURE 1. Condensed bathygrams showing double and single layers. Depth intervals, 50 fathoms. A very deep layer can be seen at sunrise and sunset in the lower figure. a. *Atlantis* cruise 152, 14 Aug., 1948; 42°44′N., 27°51′W. b. *Atlantis* cruise 152, 17 Aug., 1948; 39°15′N., 26°39′W.

dred fathoms, and this also may execute a diurnal migration. Most of the bathygrams shown here have been horizontally condensed by a photographic method described by Moore (1949c) which gives four-times horizontal reduction while retaining the vertical dimensions unchanged. In addition to allowing better presentation of the records, this method reduces the curvature of the vertical coordinates to one-fourth, thus reducing the skewing normally present in the upper parts of the bathygrams. The methods used for correcting the recorded depth of the scattering layer for the effects of depth and ping length have been referred to in Hersey and Moore (1948).

Possible sources of the scattering layer effect. There is no known physical agency which could produce an echo of the scattering layer type, with its marked diurnal vertical migration. It appears certain, therefore, that we must be dealing with a living form. The phytoplankton may be ruled out both because it does not migrate vertically and because its very small size makes it a less probable source than some of the larger forms. Sargasso weed is concentrated close to the surface, and any found at two hundred fathoms will almost certainly be sinking steadily. Further, it is absent from many parts of the ocean from which the scattering layer has been recorded. Of the animal population, we may consider separately the smaller zooplankton, and the larger and more active forms such as fish and squid. Fish, many of which have swim-bladders containing gas, offer an attractive explanation, and we know so little of their abundance in the open ocean and of their diurnal migrations, that it is difficult to evaluate their significance. However, there are certain arguments against their being the source of the scattering layer. Firstly, being predators, they must be present in much smaller total quantity than the zooplankton on which they feed. Secondly, being considerably larger than the zooplankton, they have, relatively, a much smaller surface area. Dietz (1948) also points out that many of the commoner oceanic fish which are known to inhabit the depths in question, do not have swim-bladders. Finally, all the records of assured fish shoals which we have seen have been of a very patchy and discontinuous nature, quite unlike the extremely uniform records from the organisms composing the scattering layer.

The above arguments may also be applied to Lyman's (1948) suggestion that squid may be the source of the scattering layer. Further, the geographical distribution of odontocete whales, and hence presumably that of the squid on which they feed, is not in the least like the distribution of the scattering layer in those areas of the north Atlantic which we have studied. Unfortunately the paper by Townsend (1935), on the distribution of the squid-eating sperm whales, does not include comparable data on the common north Atlantic euphausid-eating whales, so comparison of the distributions of the two types of whale with that found for the scattering layer, is not possible.

Of the zooplankton, certain groups inherently appear to be unlikely sources of the scattering layer. Subject to the results of experimental tests now in progress, it would appear less probable that an echo would be returned from an animal with a gelatinous body such as a medusa, siphonophore, or chaetognath, than from one with a more solid musculature and a chitinous or calcareous surface. Furthermore, data obtained in the Bermuda area (Moore, 1949a), do not indicate any particular concentration of these gelatinous forms at the depth of the scattering layer, the greatest numbers being considerably nearer the surface. The same is true of the forms such as pteropods and some heteropods which have a calcareous shell; in addition, most of these show a marked seasonal variation in abundance, which variation has not been observed for the scattering layer.

There remain the crustacea, which have a chitinized surface; of those crustacea which are sufficiently abundant and widely distributed to justify consideration, there are three main groups. The large red prawns (*Acanthephyra, Sergestes*, etc.) and some euphausids concentrate in the daytime at a depth of five to eight hundred fathoms (Waterman *et al.*, 1939; Leavitt, 1935, 1938) and show a definite diurnal vertical migration. These are too deep to be the source of the regular scattering

layer, but they might account for the very deep layer to which we have referred. The euphausids, usually one to two centimetres long, agree well in their distribution and behaviour with the scattering layer, and it is with these that most of the present paper is concerned. The copepods, usually only a few millimetres long, have their main concentration too shallow to be the source of the scattering layer, but they may well be concerned in reverberation. Raitt (1948) has made calculations with regard to the effective size of the organisms concerned, but we cannot yet interpret this in terms of actual physical size.

Quantitative estimate of euphausid abundance. It is necessary that the organism proposed as the source of the scattering effect be present in adequate numbers to produce the observed effect. We do not yet know the reflective properties of euphausids, although it is hoped that work now in progress will provide data on this. We are able to give some figures on the abundance of euphausids which may prove useful in future calculations. A series of net hauls was made near Bermuda at a time of night when most of the euphausids were concentrated near the surface. Oblique hauls were made at various depths from the surface down to approximately 110 fathoms, the main concentration of euphausids being at about 40 fathoms. The average catch for all depths was 273 euphausids per haul of a 70 cm. diameter net, towed for thirty minutes at 2 knots. Assuming a filtration efficiency of 50 per cent for the net, this indicates 130 euphausids in a vertical column of water one square metre in section and 200 metres deep. Those individuals below the depths sampled would probably bring this figure for the total population up to 150. The greatest concentration observed corresponded to about 1.5 euphausids per cubic metre at night, and about half this figure in the daytime.

Calculations from data of Leavitt's (1938) indicate a maximum concentration of 1.4 per cubic metre, making no allowance for a filtration factor, or 2.8 including the factor. He used larger nets (2 m. diameter), so the escape factor referred to below would probably be less in his case.

It is well known that a plankton net is inefficient in capturing the larger and more active forms, and that a considerable proportion of them are able to avoid the net. While we have no exact data on this, we have the results of night surface net hauls made at Bermuda simultaneously with visual estimates of the abundance of the brightly luminescent euphausids. From these it would appear that a net indication of only one euphausid per 75 cubic metres of water corresponded with a visual estimate of at least one per cubic metre. It would seem legitimate, therefore, to multiply our net estimates by at least ten, and probably more. This would give a value of 1500 euphausids in a column of water of one square metre cross section, extending from the surface to below the scattering layer, and maximum concentrations of 15 per cubic metre at night, 7 in the daytime. Assuming a cross section of 0.5 square centimetres for an average euphausid, when viewed from above this would mean that a vertical beam of sound would suffer at least ten per cent interception by euphausids by the time it had passed through the scattering layer, and this figure is probably too low. Finally, our estimate was made in the Bermuda area where the standing crop of plankton is comparatively poor. Nearer the continental shelf, where much stronger scattering layer records have been obtained, the zooplankton is known to be considerably richer.

Correlation between the geographical distribution of the intensity of scattering layer and that of euphausids. From our own North Atlantic and Mediterranean records, and from those of Dietz and others for the Pacific, it appears that the scattering layer is of extremely widespread, if not universal occurrence in deep water. There are not, at present, adequate data on either the quantitative geographical distribution of euphausids, or the intensity of the return from the scattering layer, to allow an overall correlation between the two. However, for the Mediterranean we have some figures which can be used (Moore, 1949b). The nutrient salts in this area decrease from west to east (Helge, 1931; Bumpus, 1948). Jespersen's (1935) figures for total zooplankton decrease similarly (Fig. 2a). Data of Ruud's (1936), which are not well suited for such a quantitative comparison, nevertheless give some indication of a similar decrease in the euphausid population (Fig. 2b). For those parts of the Mediterranean which were examined by the *Atlantis*, scattering layers were found mainly in the western area, with a few in one small area of the eastern Mediterranean (Fig. 2c). Oscilloscope records gave some support to the belief that, where the echo sounder failed to detect a scattering layer in this area, the layer was in fact weak or absent. In this area, then, there is agreement between the distribution of the scattering layer and of the zooplankton in general and euphausids in particular. We have also recorded a sharp break in the intensity of the scattering layer record at the transition point between gulf stream and northern water.

Correlation between the geographical variations in depth of the scattering layer and of the euphausids. If euphausids are responsible for the scattering effect, there should be a correlation between the day level of the layer throughout the ocean and the proportions of relatively deep- or shallow-living species forming the local population. Such a comparison is possible along a line from Bermuda to Florida and further west into the northern part of the Gulf of Mexico. The source of the biological data is given in a later section. We are probably correct in arranging the commoner euphausid species of the area in the following order of increasing average depth: *Euphausia tenera*, (*E. americana* ?), *E. brevis*, *E. hemigibba*, *Thysanopoda aequalis*, *Thysanoessa gregaria* (Fig. 19).

Figure 3 shows the area of dominance of the various species along the line in question. Their distribution suggests that, north of Bermuda, where *E. tenera* and *E. brevis* are common, the layer should be fairly shallow. Further west the deeper-living of these, *E. brevis*, becomes more abundant, and then in turn mixes with and is replaced by the still deeper *E. hemigibba* and *T. aequalis*. Around Florida *E. americana* becomes dominant, and we believe that it is a shallow form. In the Gulf of Mexico, the shallow *E. tenera* is the common form. Finally, the abundance of the usually very deep *T. gregaria* to the south of Bermuda, should mean that the layer will be particularly deep there. Figure 4, which gives the noon level of the scattering layer throughout this area, agrees well with this. The layer is shallower north of Bermuda, and much deeper towards the south. Westwards it becomes first deeper and then shallower again, and in the Gulf of Mexico some of the observations are extremely shallow. Naturally, with observations taken at different times, and over so long a period, no exact agreement can be expected, but what there is would appear to be significant.

Another test of geographical distribution is afforded by double layering. In most

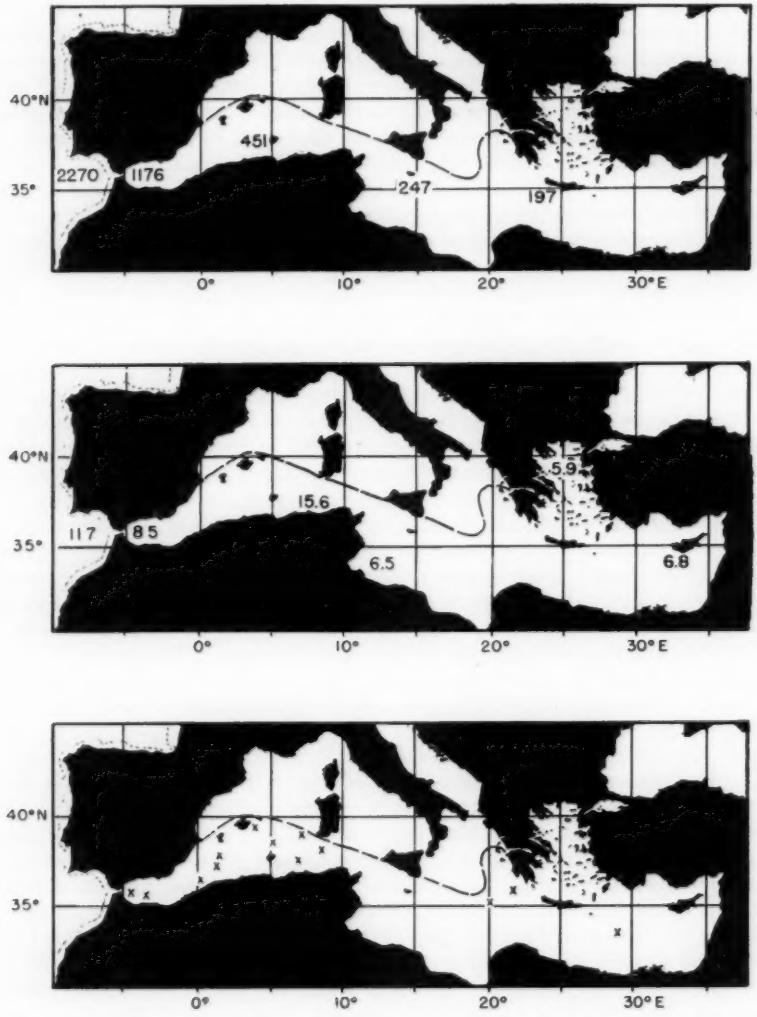

FIGURE 2. Comparison of plankton distribution and the occurrence of scattering layer returns in the southern Mediterranean Sea. a. Average volume (cc.) of macroplankton in the upper water layers (25–65 m. wire). (After Jesperson, 1935.) b. Relative numbers of euphausids per net haul in different areas of the Mediterranean. (Mod. after Ruud, 1936.) c. Distribution of scattering layer returns in the Mediterranean Sea. (No records were made north of the broken line.)

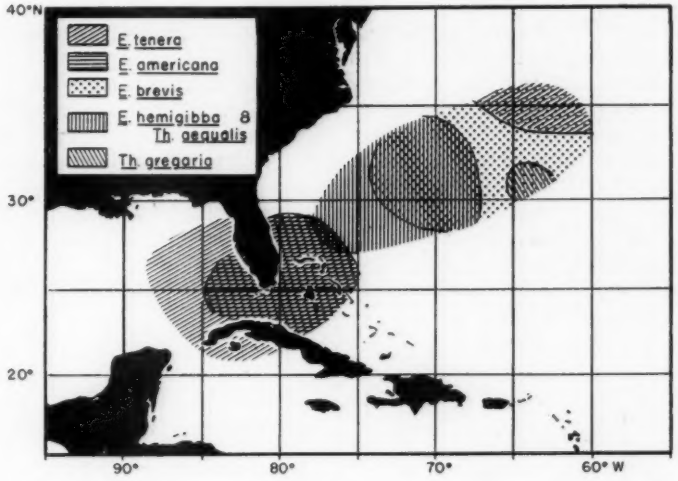

FIGURE 3. Areas of dominance of euphausid species in Western Atlantic. (Information is lacking in unshaded areas.)

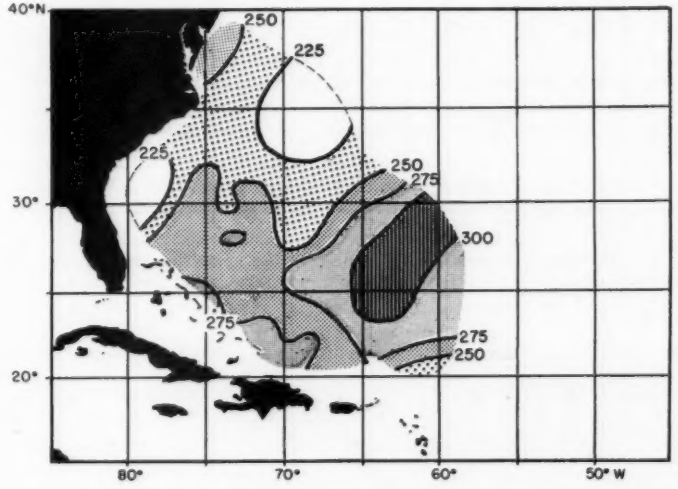

FIGURE 4. Geographical trend of the noon depth of the scattering layer. (Figures represent the corrected approximate depth in fathoms of the peak abundance of scatterers.)

areas there are several species of euphausid present, each with its particular maximum level, but also with a fairly wide vertical spread. The long ping length used tends to reduce the detail readable from the bathygram. It is not surprising, therefore, that separation into distinct layers is rather rarely seen on the present records.

There is an area to the north of the Azores where the euphausid population consists almost entirely of two species, *Euphausia krohnii* and *Nematoscelis megalops*, which appear to have markedly different vertical distributions. They occur in approximately equal numbers at some localities, while at others *N. megalops* is the commoner. South of the Azores these species are scarce or absent and the populations are mixtures of several species with less distinct vertical distributions. Figure 5 shows the data available on the euphausids of this area, and Figure 6 the locations at which very marked separation into double layers was recorded. That the separation of the two layers in the records is in fact the result of the dominance of these two species is confirmed by the fact that at one station in the western Mediterranean where the

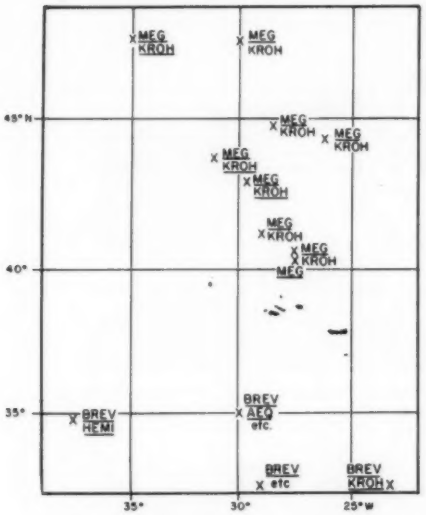

FIGURE 5. Dominant euphausids of the Azores area (most abundant species are underlined).

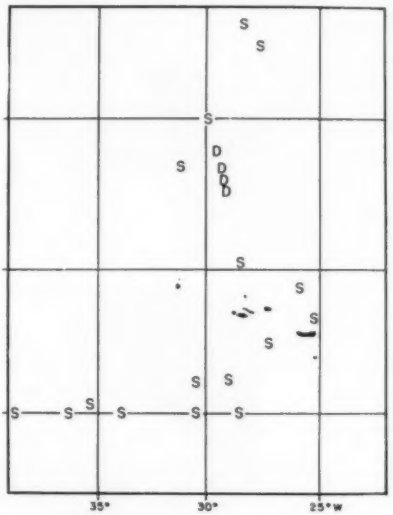

FIGURE 6. Occurrences of double and single echo in the Azores area.

same two species were dominant, similar double layering was recorded. Examples of a single and a double layer fathogram from this area are shown in Figure 1. Dietz (1948, p. 436) shows a clear example of double layering from the north Pacific.

Occurrence of the scattering layer in relation to the depth of the bottom. The oceanic euphausids are adapted to live permanently in mid-water, and do not normally ever touch the bottom. Net hauls show them to be absent from shallow inshore waters. Figure 7 shows the disappearance of the scattering layer at a point where the depth of water is slightly greater than that of the center of concentration of the layer, and similar records have been obtained where the water shallows on the edge of the continental shelf. In some inshore waters there are known to be

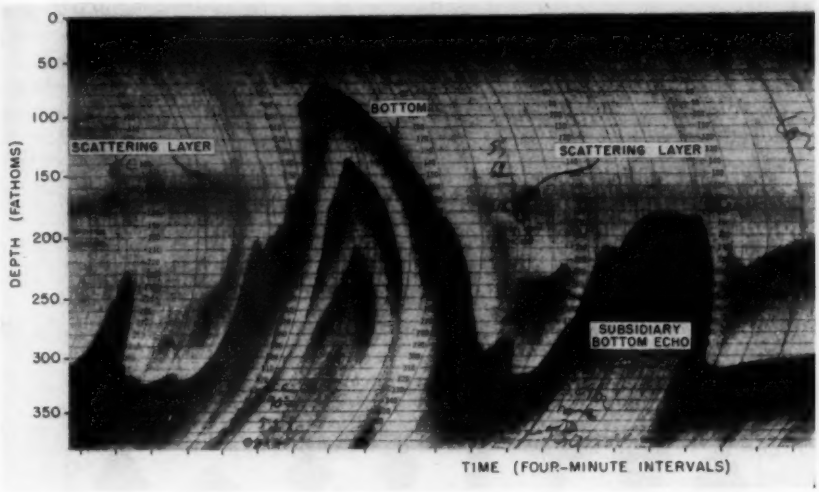

FIGURE 7. Bathygram showing scattering layer (160 to 190 fathoms) interrupted by submarine peak. *Atlantis* cruise 156, 4 June, 1949; 38°11'N., 73°30.5'W.

euphausids such as *Meganyctiphanes norvegica, Nyctiphanes couchi,* etc. which lie on, or close to, the bottom in the daytime. There are also Mysids with similar habits which may be very abundant in some inshore waters. Figure 8, which was made in water 100 fathoms deep near Miami, Florida, apparently shows such a form leaving the bottom about 40 minutes before sunset and reaching the surface just after sunset. It also shows clearly the phenomenon of downward diffusion after dark, and it should be noted that there was no moon on that occasion. Figure 9 shows a morning descent onto the bottom which, in this case, is at 200–250 fathoms.

Diurnal migration of the scattering layer and of plankton. The diurnal migration of the scattering layer is one of its most striking characteristics, and one which makes it practically certain that the source of the effect is an animal population. Discus-

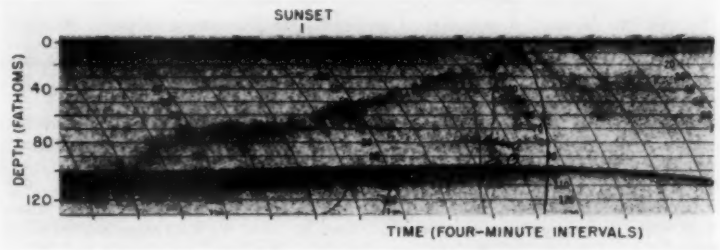

FIGURE 8. Bathygram showing evening ascent of layer from the bottom. *Atlantis* cruise 144, 11 April, 1947; 25°16'N., 80°05'W. (off Miami, Florida).

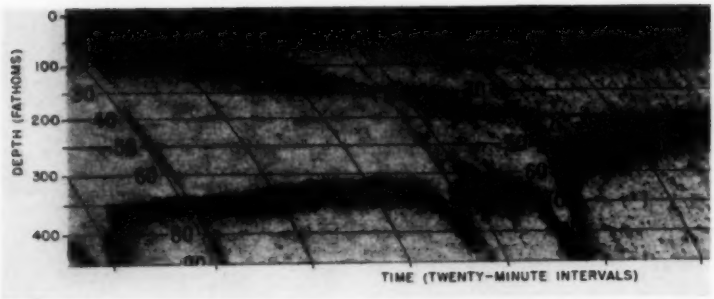

FIGURE 9. Bathygram showing morning descent of scattering layer onto bottom at 200 to 250 fathoms. *Atlantis* cruise 154, 7 Apr., 1949; 25°51′N., 79°41½′W.

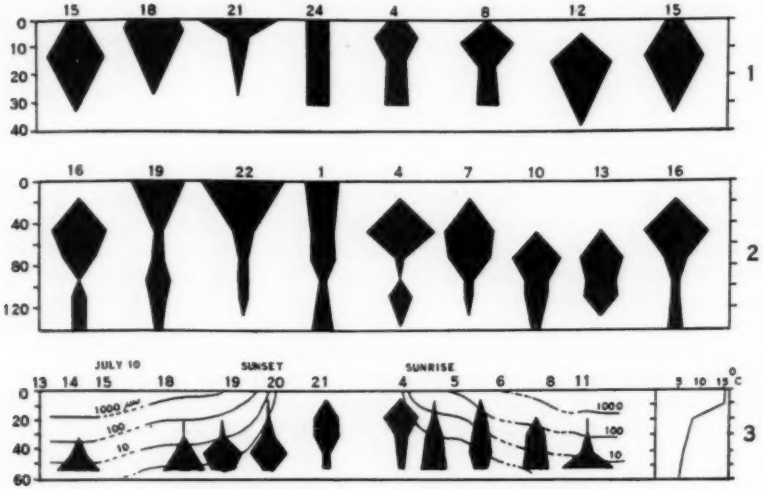

FIGURE 10. Vertical distribution of plankton animals. (From Russell, 1931.)

1. Hypothetical vertical distributions at different times in the twenty-four hours to illustrate the behaviour of a population of a species such as *Calanus finmarchicus* in its diurnal movements (after Russell, 1927, p. 237, Fig. 4).
2. Diagram showing vertical distribution of female *Calanus finmarchicus* at 3-hourly intervals during 24 hours on January 25th–26th, 1932; sunset 4.27 p.m.: sunrise 8.57 a.m. (after Nicholls, 1933, p. 150, Fig. 4).
3. Diurnal migration of adult females of *Metridia lucens* in the Gulf of Maine, July, 1932. The changes in light intensity are indicated by the lines representing the depth at which 1000, 100 microwatts etc. occurred (one microwatt may be taken as approximately equal to three metre-candles). The temperature curve at the right shows that migration right to the surface at night was prevented by the thermocline (after Clarke, 1933, p. 426, Fig. 5).

sion of some deductions made from bathygrams is deferred to the later section on the biology of euphausids, but some of them require consideration here.

Russell (1931) has presented a hypothetical pattern of diurnal variation in vertical distribution for a copepod, *Calanus finmarchicus* (Fig. 10). This shows a sequence of upward movement around sunset, followed by a general diffusion during the dark period; some upward concentration again immediately before sunrise, and descent to the day level when the sun rises. He also gives a graph of actual distributions observed by Nicholls (1933) for the same copepod which very closely paral-

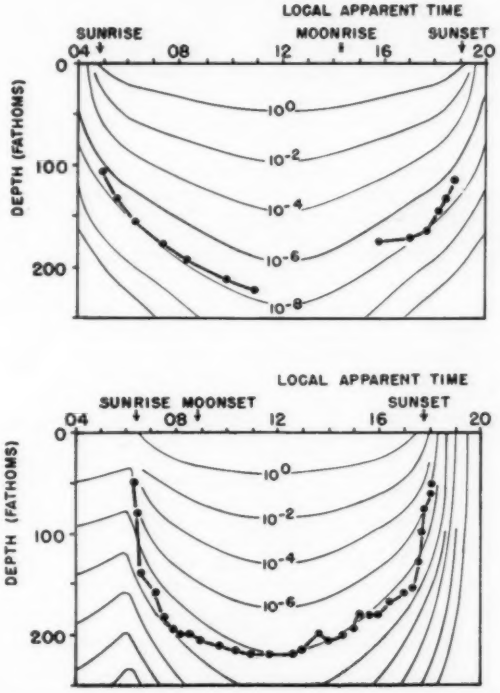

FIGURE 11. Diurnal movement of scattering layer and of iso-illumination curves. a. *Atlantis* cruise 151, 18 May, 1948; 38°51'N., 03°27'E. b. *Atlantis* cruise 144, 8 Feb., 1947; 25°07'N., 92°35'W.

lel his own hypothetical figures. The figure of Clarke's (1933) for the same species, which Russell gives, has iso-illumination curves superimposed, and it is clear that the migrations tend to follow the iso-illumination curves. In Clarke's observations, however, the warm water at the thermocline appears to form a barrier which limits upward migration. A figure showing the diurnal migration of euphausids is given in Hersey and Moore (1948).

Illumination is the chief factor motivating the upward and downward migrations, but, as shown later, limiting temperatures may form barriers at the two ends of the

migration. Figure 11 shows records of the migrations of the scattering layer, taken from bathygrams, which closely parallel the curves of iso-illumination. Figure 12 shows that the layer may migrate in response to moonlight as well as to sunlight. Dietz (1948) has shown that the scattering layer was lost when he entered Antarctic waters during the period of continuous daylight, though its reappearance showed

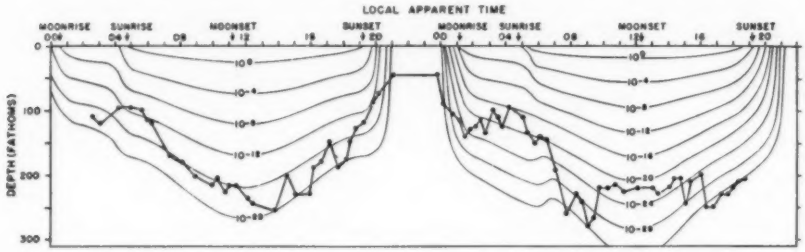

FIGURE 12. Diurnal migration of scattering layer showing, on second day, two periods of descent, one at moonrise and the other at sunrise. Deepening of layer in latter half of second day was associated with an increase in water temperature. *Atlantis* cruise 141, 23–24 May, 1946; noon locations: 38°26′N., 74°59′W. and 36°17′N., 74°41′W., respectively.

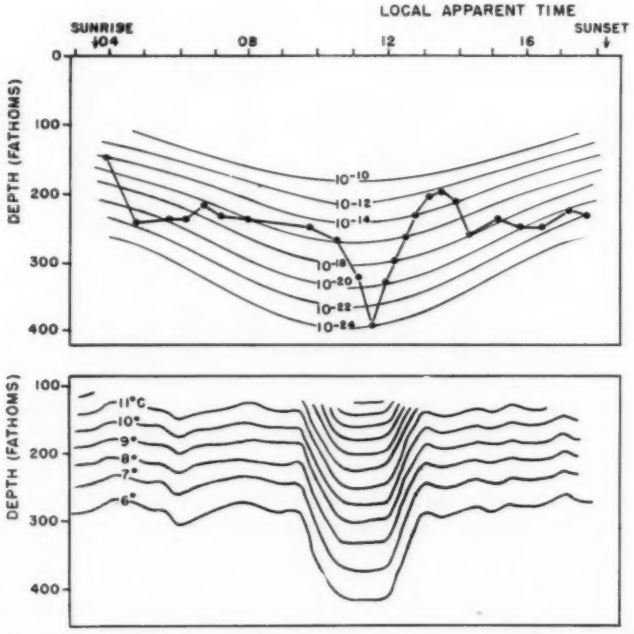

FIGURE 13. Diurnal migration of scattering layer showing its relation to temperature and illumination. *Atlantis* cruise 149, 1 July, 1947; 36°59′N., 66°57′W. a. Scattering layer and iso-illumination curves. b. Isotherms.

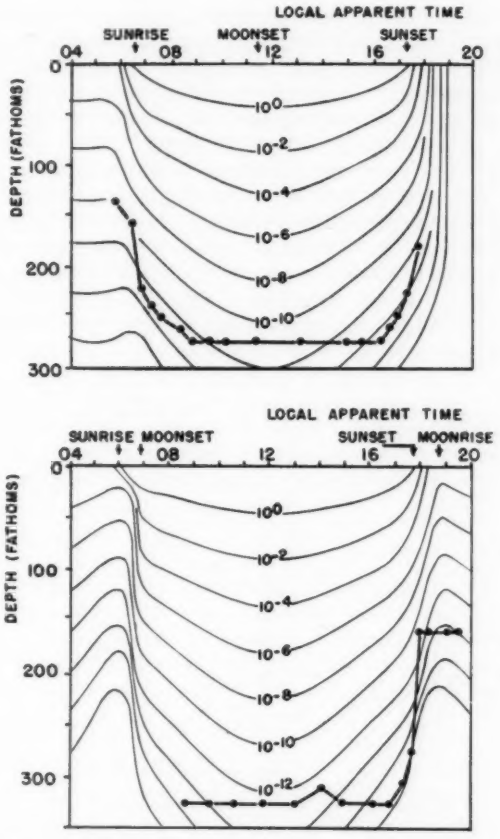

FIGURE 14. Diurnal migration of scattering layer showing marked noon flattening. a. *Atlantis* cruise 144, 13 Jan., 1947; noon position: 21°21'N., 75°39'W. b. *Atlantis* cruise 143, 11 Oct., 1946; noon position: 26°04'N., 60°55'W.

only approximate agreement with the return of the ship to an area of alternating day and night. He compares this effect with Bogorov's observations (1946) on the cessation of diurnal migration of the plankton in the Arctic during the summer period of permanent daylight. The disappearance of the scattering layer suggests, however, that in addition to ceasing to migrate, the organism responsible for the scattering effect becomes too diffuse vertically to show as a distinct layer in the bathygrams.

Relation of the depth of the scattering layer to temperature. Since the specific constitution of the zooplankton varies geographically, and different species have different optima and limits for temperature and other factors, no simple, overall

correlation between the depth of the scattering layer and temperature can be expected. In a limited area where the plankton constitution is known to be more or less constant, such a correlation might be sought. Figure 13 shows a record of the variation in depth of the layer on a day when the *Atlantis* passed abruptly into much warmer water and back (the edge of the Gulf Stream). The coincidence of the sudden deepening of the layer and the warmer water is clear. The record was made on the edge of an eddy of cold water trapped on the Sargasso Sea side of the Gulf Stream. In another case, the coincidence of this change in depth of the scattering layer with the observed temperature changes in the water was so good that it was possible to make use of it in mapping the warm-cold interface. Unfortunately we cannot be sure, in these cases, that we were dealing with a similar zooplankton community in the cold and warm masses of water. In the case of most bathygrams from the open ocean we can be fairly certain that we were dealing with a similar zooplankton population throughout any one day.

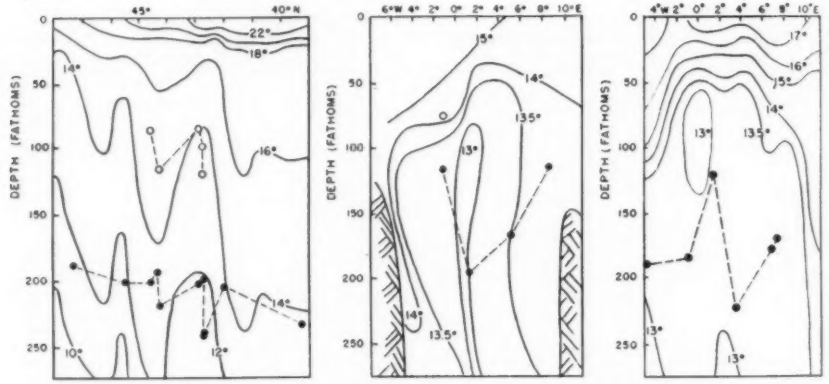

FIGURE 15. Noon scattering layer depths and temperature profiles. (Azore temperatures modified from Iselin, 1936; Western Mediterranean temperatures from Bumpus, 1948.) a. Azores area (July–Aug.). b. Western Mediterranean (Jan.). c. Western Mediterranean (Apr.–May).

The evidence so far presented shows a rather widespread similarity between the distribution and behavior of the scattering layer and those of euphausids. Assuming, for the moment, that the two are connected, we may examine the effects of temperature on their diurnal migration. Figure 14 shows two bathygrams in which the marked mid-day flattening of the scattering layer is interpreted as meaning that the euphausids met, at that depth, a cold barrier which interrupted their descent. We have already referred to the area in the neighborhood of the Azores in which the two observed scattering layers are believed to be produced by the two dominant euphausids *Nematoscelis megalops* and *Euphausia krohnii*. Figure 15a shows the noon depths of these two layers on a transect through the area, together with the temperature profile.[3] The more complete lower, or *Nematoscelis* layer shows an

[3] The scattering layer observations, and those on which the isotherms are based, were made on different cruises, so exact agreement cannot be expected.

increase in depth paralleling the increase in temperature southwards. The two graphs shown in Fig. 15b and c were obtained from data from the area in the western Mediterranean where, again, a *Nematoscelis* layer could be distinguished. While the Azores series showed considerable noon flattening, which we interpreted as indicating the presence of a cold barrier to descent, the Mediterranean series showed little or none. It will be seen that in the Mediterranean, the *Nematoscelis* are living, on the whole, in warmer water than in the Azores area, and that they are in a zone with almost no thermal stratification. The generally shallower depths of the layer in the Mediterranean are in agreement with the higher extinction coefficients and lower illuminations relative to the Azores area. The fact that, in these areas where we believe that we are dealing with a single species of euphausid only, the occurrence of noon-flattening is dependent on low temperatures and thermal stratification, together with the dependence of the depth at which this noon-flattening occurs on temperature, gives considerable support to our temperature-barrier theory.

The fact that such a relationship emerges in an area where we know that we are dealing with an unusually simple euphausid population is another argument in favor of euphausids being responsible for the scattering effect. The evidence would, of course, be stronger if we had tow nettings made in the layer at all these stations, but this was not possible. Where we have taken tow nettings in the layer in other areas, we have found the main concentration of euphausids to be at the depth indicated by the bathygrams (Hersey and Moore, 1948).

Other types of scattering layer. So far we have discussed only the daytime distribution of the main scattering layer. While we have some night records, more will be needed before any analysis can be made. We have referred briefly to two other types of scattering layer, the very deep one, and one which apparently shows a reversed diurnal migration. A few complete day records of the deep one have been

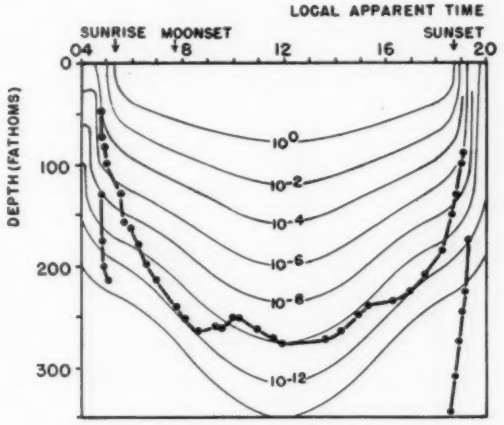

FIGURE 16. Diurnal migration of scattering layers, showing main layer throughout day and very deep layer around sunrise and sunset. *Atlantis* cruise 152, 22 Aug., 1948; noon position: 37°29′N., 27°18½′W.

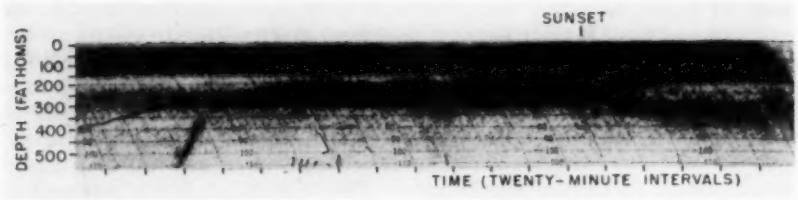

FIGURE 17. Bathygram showing evening ascent of one scattering layer and the simultaneous descent of another. *Atlantis* cruise 149, 7 July, 1947; noon position: 38°42'N., 69°32'W.

obtained, but more often the layer is indicated on the bathygram only around the periods of ascent and descent, and tends to be missed at its greatest depths (Figs. 1b, 16). It apparently performs a diurnal migration very similar to that of the main layer, but descends slightly earlier, and ascends slightly later. Its night level also appears to be deeper than that of the main layer. The day level is much deeper than that of the main euphausid concentration, and it is suggested that the organisms responsible may be the large Acanthephyrid and Sergestid prawns. Waterman *et al.* (1939), and Leavitt (1935, 1938) have shown that there is a concentration of these prawns and of certain euphausids at about the required depth. The prawns are considerably larger than the euphausids, but there is little information on their abundance. Being larger and more active than the euphausids, they are even less likely to be adequately sampled by nets, so it is quite possible that they occur in considerable numbers.

Bathygrams frequently show a record similar to that in Figure 17 in which, immediately after the evening ascent of the main layer, what looks like a continuation of this layer remains on at the same level or descends slightly. In Hersey and Moore (1948) an oscilloscope analysis of such a condition was given. The much greater detail possible with short ping length and oscilloscope recording, shows that there were, in fact, two layers, a deep one which ascended in the normal way at sunset, and a shallow one which descended and crossed it. The long ping length generally used on the echo sounder would obscure the details in such a crossing over and give a record similar to that shown in Figure 17. With only one series of oscillograms, it

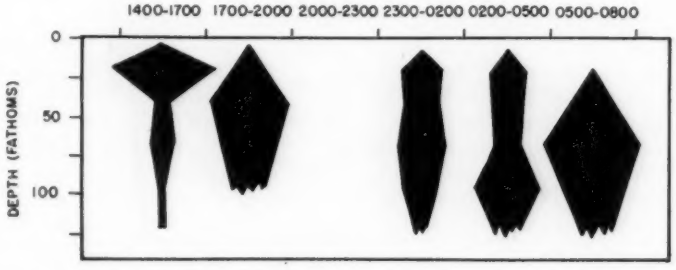

FIGURE 18. Diurnal migration of *Stylocheiron abbreviatum* and *S. carinatum* combined. Bermuda, 4–5 Feb., 1949.

cannot yet be said whether this is always the explanation of such records, but there is no apparent reason why it should not be.

Not much is known about plankton forms which show this reversed type of diurnal migration, although several workers have suggested the possibility. In Moore (1949b) it was shown that there was a correlation between the day level inhabited by various species of zooplankton and the extent of their diurnal migration. The greatest migration was shown by the deepest-living forms, while in shallow-living forms there was little or no migration, and in a few of the shallowest forms there appeared to be a downward movement at night. This statement is based on a number of day hauls but on only one series of night hauls, and further series are needed before we can be sure about the downward night migration. Among the euphausids, some of the species of the genus *Stylocheiron* appear to exhibit such a night descent (Fig. 18). The numbers taken were too few to prove the point, but it is tentatively suggested that some members of this genus and in particular *S. abbreviatum* and *S. carinatum*, might be responsible for the recorded night descent. It is, of course, quite possible that in this case some form other than a euphausid is responsible.

Euphausid Biology

Geographical and vertical distribution. Information on the oceanic distribution of planktonic forms, both vertical and geographical, is very incomplete, and unfortunately this is particularly true for euphausids. Einarsson (1945) gives much information for the northern part of the North Atlantic, but makes only brief reference to the more southern species considered here. Ruud (1936) gives considerable data on geographical distribution of euphausids in the Mediterranean, and a little on the relative vertical distributions of the species. Tattersall (1926) covered a good deal of the area between Bermuda and the American coast, and from about 23° to 37°N. Leavitt (1935, 1938) and Waterman *et al.* (1939) give good vertical distribution data at a few stations in the same area, and these, together with our own from near Bermuda (Moore, 1949a), are practically our only source of information on vertical distribution. The *Atlantis* has, for a number of years, taken night surface tow nettings throughout the North Atlantic, Mediterranean and Gulf of Mexico. Descriptions of these have not been published, but are made use of here. Information on diurnal migration is taken from Waterman *et al.* (1939) and from Moore (1949a).

For geographical distribution, we have considered all hauls in which an adequate number of euphausids was taken, and expressed each species as a percentage of the total euphausid population. This was necessitated for comparison of various types of hauls. In some cases either oblique hauls, or a series at different depths, yield a good sample of the population at all depths. Daytime hauls near the surface are likely to be poor in representatives of the deeper species. For the sake of conserving ship's time, we have made use largely of surface hauls taken around midnight. At that time the euphausids will have approached the surface, and tests at Bermuda showed that a fairly representative sample of all but the deepest forms was obtained. Since these night hauls form a large proportion of our data, and since we have reason to suspect that some species of the genus *Stylocheiron* descend at night, this whole genus has been omitted in calculating percentages. The combination of such mixed data is only justified by our need for information from a wide area, and the distri-

bution patterns obtained must be considered significant as a whole rather than in detail.

From observations at Bermuda, which are summarized in Figure 19, and from the results of Leavitt (1935, 1938) and Waterman *et al.* (1939), we are probably correct in arranging the common species of the area in the following order of increasing depth of day level: *Euphausia tenera, E. brevis, E. hemigibba, Thysanopoda aequalis, Thysanoessa gregaria.* Ruud (1936) confirms the fact that *E. hemigibba* lives deeper than *E. brevis,* and that *T. gregaria,* though very variable, is a deep form. *E. americana* is believed to be a shallow form, comparable with *E. brevis* or *E. tenera.*

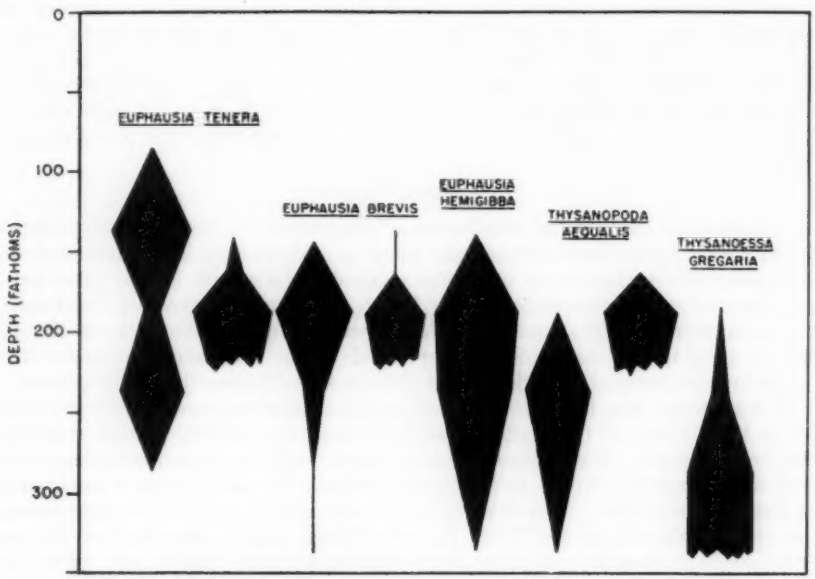

FIGURE 19. Vertical distribution of the commoner species of euphausids off Bermuda, from hauls made in the middle of the day.

E. krohnii also is probably comparable with *E. brevis,* and *Nematoscelis megalops* is deeper. *Stylocheiron* spp. rarely form a large proportion of the catches. Some of them are very shallow species, and so were taken in a sufficient number of Bermuda hauls to yield fairly accurate knowledge of their distribution. Of these, *S. suhmi* has a mean day level of 25 fathoms, *S. carinatum* of 44 fathoms, and *S. abbreviatum* of 57 fathoms.

Temperature and illumination—sources of data. Temperature data have been obtained from the nearest hydrographic station, supplemented where needed by the shallower but more numerous bathythermograph records. The latter were made at the same time as the scattering layer and net haul records, as were those at the

hydrographic stations in the Mediterranean. In other areas older hydrographic information had to be used, but at the fairly stable depths with which we are concerned, this is not likely to give rise to much error except in areas of large fluctuation such as the margins of the Gulf Stream. The source of illumination data is discussed in the Appendix.

Diurnal migration and the environmental factors involved. There seems little doubt that illumination is the major factor in controlling diurnal migration, but the reaction of the animal to light is not simply one of moving so as to remain at a constant illumination. While the only known factors which ordinarily can produce a directional effect are light and gravity, the response of the animal to these can be

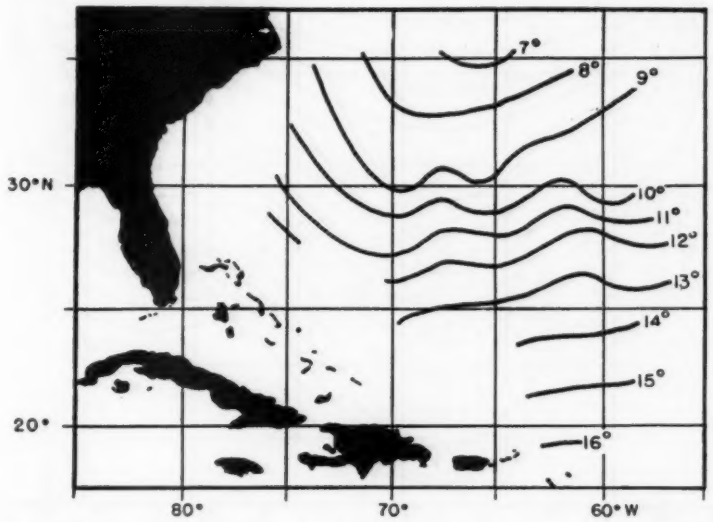

FIGURE 20. Difference in temperature (°C.) in summer between the surface and a depth of 328 fathoms.

modified by other factors such as temperature. Kikuchi (1930) has reviewed the factors which are so far known to affect diurnal migration. These may include internal factors as well as external factors so that different levels may be characteristic of males, females, and immature individuals of the same species. Russell (1931) points this out, and in another paper (1927) he refers to cases in which increased temperature may make a previously positively phototropic form negative, while cooling may have the reverse effect.

The environment of animals living as deep as the euphausids differs in many ways from that of shallower forms, and some of the differences are not obvious at first sight. To begin with, in the course of their diurnal migration they will undergo considerable changes of temperature. Figure 20 indicates of what order these may be for euphausids in one section of the north Atlantic. Such changes must have very

considerable effect on the physiology of the animals. They will also undergo a change in pressure of 50–60 atmospheres. Pressure is a factor which has received little consideration except in so far as it affects much deeper-living forms. Some results obtained by Hardy and Paton (1947) suggest that it may be operative, even at lesser depths. Reynard (1885) subjected various shallow-living forms to increasing pressure and found that they became at first more active, and then, with further increase, quiescent. From the summaries of Cattell (1935, 1936) it would appear that in general, and considering only comparatively low pressures, increased pressure on nerves increases their action potential and rate of propagation,

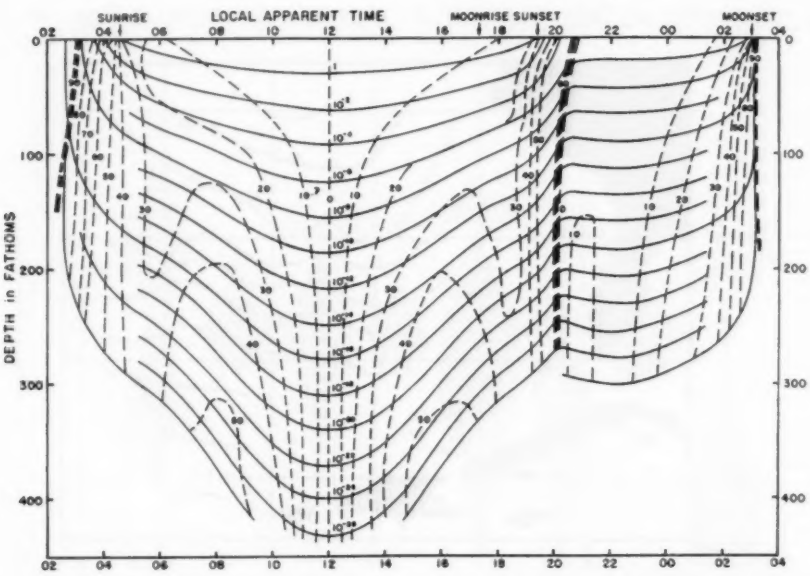

FIGURE 21. Hypothetical diagram illustrating diurnal changes in illumination at different depths. Iso-illumination curves (whole lines) show values related to a figure of 100 for overhead sunlight at the surface. Physiological stimulus (broken lines) is shown as curves of equal rate of change of illumination in arbitrary units.

and decreases their threshold of stimulation. In the case of striped muscles, above a critical temperature, increased pressure increases their contraction, while below that temperature it decreases it. Pressure changes of the order involved in a migration of three hundred fathoms might be expected to produce significant changes of this type.

Conditions with regard to illumination will be different in several ways from those for shallow-living forms. The level of illumination will be very much less, although Waterman *et al.* (1939) point out that there should be enough light to stimulate the eyes of crustacea at a depth of about 600 fathoms in the open ocean. If we refer to the iso-illumination curves (whole lines) in Figure 21 it is apparent

that illumination changes are very different for shallow and deep forms. Assuming for the moment that the plankton migrates vertically so as to maintain itself always at its optimum illumination, then an animal whose day level is less than 100 fathoms will, in the example shown, be able to ascend right to the surface at night and still find less than optimum illumination, despite the fact that there is a nearly full moon. An animal whose day level is at 300 fathoms will find

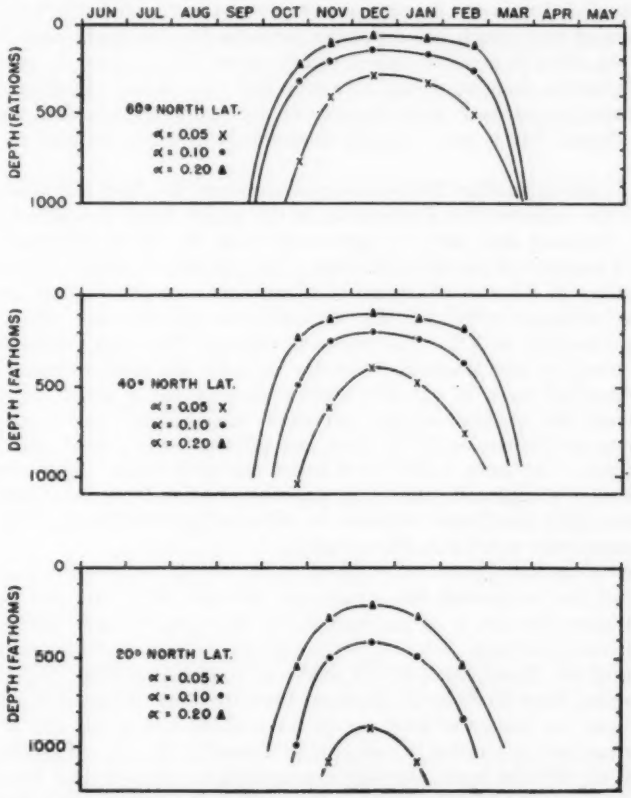

FIGURE 22. Relationship of depth at which illumination from full moon at transit equals that from noon sun with latitude, season, and extinction coefficient. (The depth is least at the moon's northernmost and the sun's southernmost transit.)

similar illumination during much of the night at about 150 fathoms, and would, at the surface, find illumination about 10^{13} times brighter than its optimum. Furthermore, in the northern hemisphere at full moon in winter, and when there is the maximum difference in declination between the sun and moon, the lunar rays will have so much shorter a path to travel in reaching a given depth than the solar rays, and their vertical absorption rate will therefore be so much less, that there should

be a depth at which moonlight will be brighter than noon sunlight. It is realized that in making this statement no allowance has been made for the scattering of the light, and it may be that the scattering will mask this effect, but if our argument in the Appendix on the importance of scattering in oceanic water is valid, the point seems worth considering. Figure 22 shows the calculated depths at which full moonlight will equal noon sunlight, and the variation of these with season, latitude, and extinction coefficient. Even if scattering largely masks this effect, it is clear that for animals living in the daytime at a depth of a few hundred fathoms there is, at some times of year, much less difference between day and night than for surface forms. If the effect is genuine, and it would appear that it must be so at least in very high latitudes, then we would expect to find the curious situation of shallow forms migrating upwards at night because of decreasing illumination, while at the same time deeper forms were moving downwards at night because of increased illumination.

Figure 21 shows another difference in environment for deep and shallow forms. Suppose for the moment that illumination is the major factor concerned in diurnal migration. Suppose also that, in agreement with the Weber-Fechner law, the response is a function of the rate of change of the stimulus. Then if we plot curves for equal values of the rate of change of illumination we should have an index of the predicted response in the animal. Consider two animals, one with a day level at 50 fathoms and one at 300. The response values will be high for the deep-living form for a much greater fraction of the day than for the shallow one. In fact, if there is a threshold value of, say 20 (arbitrary units), below which no response is to be expected, the shallow animal will show response for only a short period around sunrise and sunset, while the deep one will have only a brief mid-day period of no response. The same is true to a lesser extent at night. It is obvious that the migrations of a euphausid cannot be explained as simply as this diagram might suggest, since both non-linear response to stimulus and modifying effect of other varying factors must enter into the complex.

Analyses of the bathygrams from the point of view of temperature and illumination have not yet progressed far enough for generalization, but two records are shown in Figure 23a and b as an example of the type of result which is found. The movement of the layer is followed throughout the day, and the temperature is plotted against the illumination at the time and level in question. In Figure 24a there is a period from 04.30 to 05.40 hours when the illumination is increasing, but the layer shows no change of level except for a slight rise at the end of the time. Such a rise just before sunrise is indicated in Russell's diagram (Fig. 10). At an illumination of 10^{-8} the layer abruptly commences to descend, and does so at an accelerating pace until 07.00. During this period it is descending so fast that, despite the fact that the sun's altitude is increasing, the layer is able to attain levels of lower illumination. At an illumination level of about 10^{-12}, descent slows down so as to follow a level of constant illumination for a time, with a gradual transition to a short noon flattening period in which illumination rises, but descent ceases and the temperature remains constant. In the afternoon a similar sequence is followed, but with some hysteresis so that a path of lower illumination is followed throughout.

Figure 23b shows an example in which the noon flattening is more pronounced. The upper levels are missing from this record, but the rest of the pattern is the same except for the sharper break at 08.55. Here the period of decreasing tem-

perature but constant illumination changes over abruptly to one of constant temperature but increasing illumination. There is again a lag in the evening ascent, and here also the change at the commencement of ascent is more abrupt. The relation of these observations to what is known of zooplankton tropisms will not be discussed until more analyses have been made, but two points in particular may be referred to. The hysteresis between the curves for ascent and descent means that the trace of the scattering layer movements will not be quite symmetrical about a midday axis. Also, the fact that the organisms are able to ascend and descend faster than to the iso-illumination curves means that the speed necessary to keep pace with changing illumination is well within the capacity of the animals concerned. Maximum recorded speeds are of the order of one to two fathoms per minute which does not seem excessive for as active an animal as a euphausid. Records from

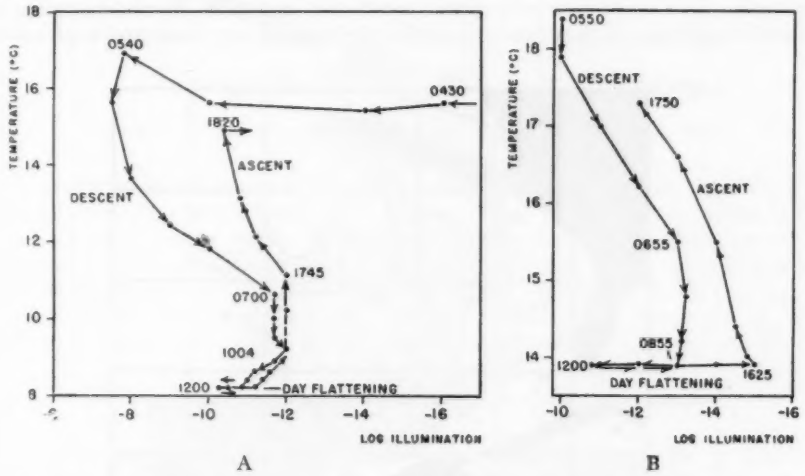

FIGURE 23. Conditions of temperature and illumination experienced by a scattering layer in the course of its diurnal migration. (Figures show local apparent time.) a. *Atlantis* cruise 144, 24 Feb., 1947; 25°26'N., 93°44'W. b. *Atlantis* cruise 144, 13 Jan., 1947; 21°21'N., 75°39'W.

Bermuda (Moore, 1949a) show that the much smaller copepod *Pleuromamma abdominalis* can ascend at a rate of about one fathom per minute.

It should be kept in mind that in referring to the effect of a factor on rate of ascent or descent, we do not necessarily infer that the rate of swimming is modified. The mechanism might equally well be one in which an organism, while maintaining a uniform rate of swimming, varied the degree of directness of its somewhat crooked course under the influence of the factor. This may be an important distinction if an attempt is made to explain observed behaviour in terms of experiments on tropisms.

When the euphausids ascend towards the surface at night, it is reasonable to suppose the warmer water encountered may constitute a barrier, just as the cold water did for the day descent. We do not yet have sufficient data on night levels

to confirm this, but the sharp night levelling-off in some of the few available records suggests this (cf. Fig. 12). Unlike the day condition, illumination and temperature will, at night, offer barriers in the same direction (that is, to further ascent) and so will not conflict. Since both the phase of the moon, and hence the illumination from it, and also the duration of lunar illumination fluctuate so rapidly, it is probable that any long-term effect such as geographical distribution will reflect surface temperatures more closely than it will night illumination. This is not, of course, true of short-term effects such as the record of migration during a single night. Maximum summer surface temperatures, in so far as they reflect the actual temperatures of the water at the night level of the scattering layer, may therefore be considered as a likely factor limiting the geographical distribution of the species.

Limiting factors in geographical distribution. Since the phenomenon of the scattering layer is of such widespread occurrence, and since its understanding is of such importance in underwater acoustics, the value of any theoretical explanation

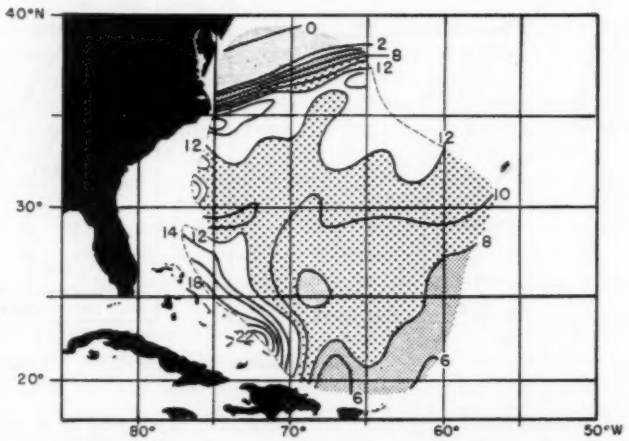

FIGURE 24. Illumination in summer at depth of 16° isotherm.

will be greatly enhanced if it allows prediction of the occurrence and nature of the layer in a given locality. It was found in the Mediterranean that there was some correlation between the intensity of the scattering layer and the density of the euphausid population. It was also found, in a limited area of the Western Atlantic, that there was agreement between the depth of the layer and the dominance of deep- or shallow-living species of euphausids in the population. More extensive data are, of course, needed before either of these points can be considered proven. In this section, an attempt is made to show that the regions of dominance of the various species of euphausid may be defined in terms of the environmental factors of temperature and illumination.

The limiting factors concerned in the mid-day levels of the euphausids appear to be a combination of illumination and temperature. If, on account of either low extinction coefficients or low latitudes, illumination is relatively high, then an animal

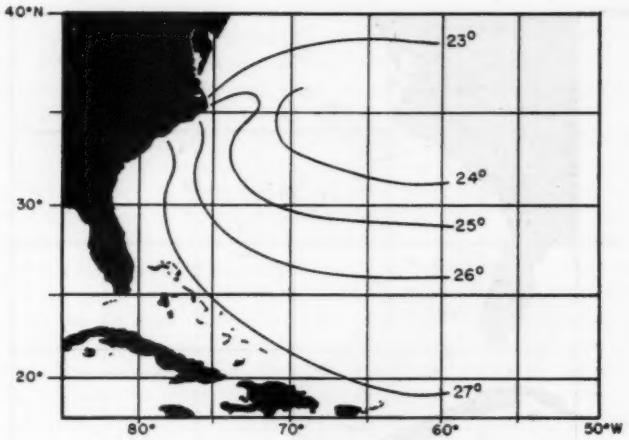

FIGURE 25. Surface temperatures in June. (Mod. from Fuglister, 1947.)

will tend to be forced down into deeper and colder water. Alternatively, if the cold water is near the surface, it may be kept up at a level where the illumination is too high. It may thus be impossible for the animal to find a level where both temperature and illumination are within the tolerance range of the species, and the area must therefore be considered unsuitable for it. In an attempt to express the combined effect of these two factors, we have made use of the illumination at midday, in summer, at the depth of the 16° C. isotherm. This isotherm was chosen

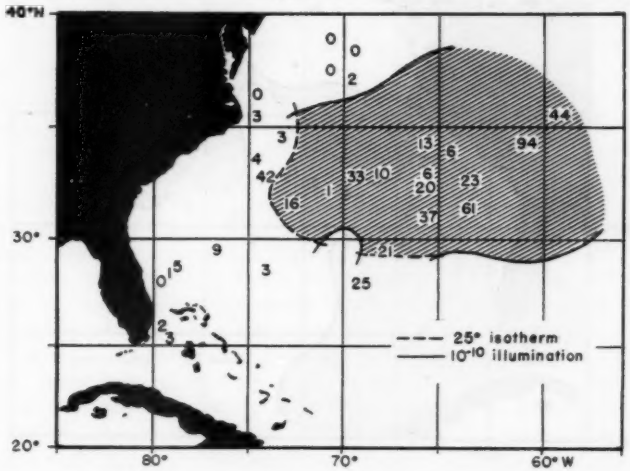

FIGURE 26. Observed distribution of *E. brevis*, and proposed temperature and illumination boundaries.

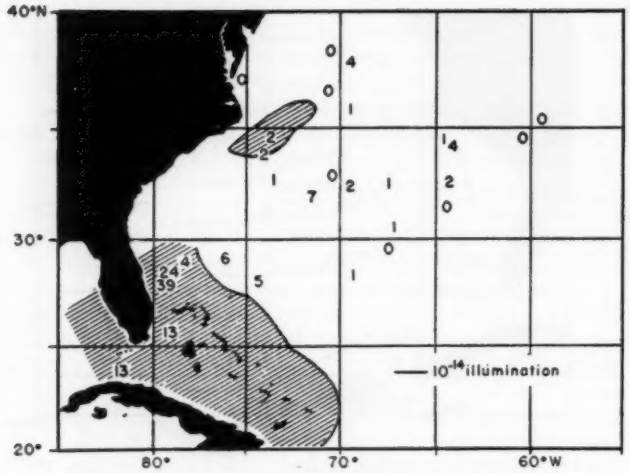

FIGURE 27. Observed distribution of *E. americana* and proposed boundary of illumination only.

arbitrarily, but, throughout the area of the Northwestern Atlantic which we are considering, it lies at about the depth of the scattering layer. Information on the transparency of the water in the area is scanty, so the chart of illumination at the 16° C. isotherm, shown in Figure 24, is subject to the reservation that it may have to be modified when better data become available.

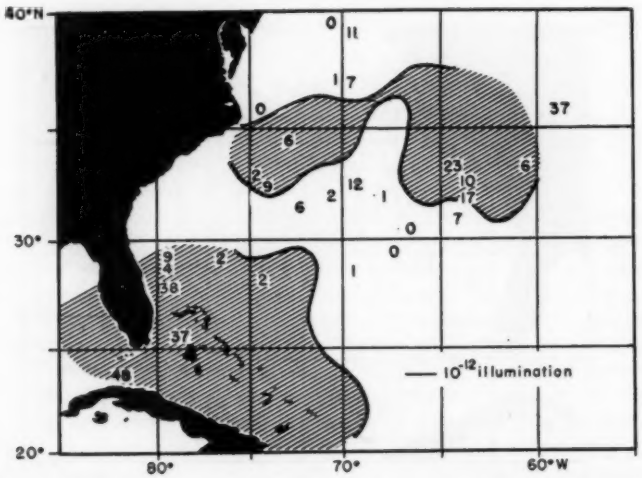

FIGURE 28. Observed distribution of *E. tenera* and proposed boundary of illumination only.

We have also suggested that at night, when the euphausids are near the surface, they may encounter too high a temperature and be prevented thereby from ascending into the phytoplankton-rich upper waters to feed. The highest temperature encountered will be in summer, and we have used the summer surface isotherms shown in Figure 25 as an index of these surface conditions.

If our choice of limiting factors is correct, it should be possible to select contours from these two charts which will define the areas inhabited by the various species of euphausids. It must be remembered that, in both cases, the curves represent one-way barriers. Surface isotherms represent a limit to spreading towards warmer, but not towards cooler water, while iso-illumination curves represent a barrier to spread into brighter conditions only (lower indices). Figures 26 to 28 show the centers of distribution of three of the commoner species of euphausid in the area, as far as we know them at present, and the values of illumination and temperature which appear to delimit them. The agreement is reasonably good. Data are now being collected from the whole North Atlantic, and the results so far obtained seem to bear out the validity of our choice of limiting factors. If, in this wider area, equally good agreement is obtained between the depth of the scattering layer and that characteristic of the dominant species of euphausid, it is likely that prediction of the depth of the layer for any given locality may be possible.

Summary

The source of the scattering effect is not, at present, susceptible of direct proof, but if an organism could be found whose characteristics agreed with those of the layer on a sufficient number of points, then there would be a high probability that this is the actual source of the effect. From the diurnal migration of the layer there seems no room for doubt that it is caused by an animal. Of the animal population of the sea, three groups seem considerably more probable than any others, namely, fish, squid, and crustacea. There are certain arguments which make the latter the most likely, and if experimental tests now in progress show that the crustacea meet the acoustical requirements, then there will be little reason to consider either fish or squid.

Of the crustacea, the small copepods may prove to be the source of shallow reverberation and possibly some very shallow layers. Euphausid shrimps are the dominant crustacean at the depth of the main scattering layer. These and large red prawns are the dominant crustacea at the depth of a still deeper layer which is briefly referred to. Euphausids occupy the same day level as the main layer, and execute a similar diurnal migration. The dominant species of euphausid varies geographically, and each species has a characteristic day level. Where it has been possible to make a comparison, there is reasonably good agreement between the geographical trend of the daytime depth of the layer and dominance of deep- or shallow-living euphausids. The euphausid population is generally a complex of a number of species. The recording instrument used does not give sufficient definition to show a number of separate layers under such conditions. Certain areas were examined where there was a simpler euphausid population with only two abundant species, and these had different characteristic levels. The bathygrams from this area showed two distinct layers on a number of occasions. The scattering layer is usually confined to deep water, and disappears or is replaced by one with

different characteristics in shallow water. This parallels the distribution of oceanic euphausids, but would probably apply equally well to other oceanic animals. The same is true of the correlation found in the Mediterranean between the decrease eastwards of the scattering effect and the similar decrease of the total plankton, including euphausids. The relation found between the level of the scattering layer and illumination, and the modification of this by temperature, is that which would be predicted for euphausids, but also for other planktonic animals. In one type of scattering layer, a reversed type of diurnal migration has been recorded, the layer descending in the evening and ascending in the morning. It is believed that certain euphausids behave in this way. Such reversal is rare in zooplankton and we do not, at present, know of any other organism which makes as extensive a reversed migration as this particular layer. The characteristics required in an organism which could be the source of the scattering effect are met, in all cases in which they have been tested, by euphausids. They are met in only some cases by other planktonic forms. There seems, therefore, to be a considerable probability that it is euphausids which cause the effect.

In the section on the biology of euphausids, a summary of relevant information for the North Atlantic area is made. On the basis of analyses of the bathygrams, a theory is proposed to explain the limiting action of temperature and illumination as environmental factors. It was found that, subject to the limitations of data at present available, the geographical distributions of various euphausid species, as predicted from hydrographic observations, agreed fairly well with that observed in net hauls. Most previous work on zooplankton has been confined to the shallower-living forms, so attention is drawn to the considerable differences in environmental conditions to which such deeper-living forms as euphausids are subject.

Actual measurements of illumination at these depths have yet to be made. The attempt to compute the illumination and its changes under different conditions is discussed in the Appendix.

At one time and another very many of the staff of the Woods Hole Oceanographic Institution have assisted in obtaining fathograms and other sonic data, hydrographic data, and plankton hauls. Mr. Iselin, Dr. Redfield, Dr. Hersey, Dr. Clarke and others have constantly helped with advice and suggestions. Mrs. Bunker has made many of the calculations and euphausid counts, as well as assisting in the preparation of the paper. To all of these I wish to express my great indebtedness.

Appendix

Illumination computations. Photocell measurements have not been made at as great a depth as that of the scattering layer, so it has been necessary to calculate the illumination there. In doing this certain assumptions have been made for lack of data, and others for simplicity of calculation. An arbitrary value of 100 for overhead sunlight immediately above the water surface, has been taken. This is equivalent to about 10,000 foot candles. In calculating the diurnal changes at a given date and locality, allowance has been made for the effect of varying zenith distance of the sun on the illumination of a given horizontal area. Allowance has also been made for the varying loss by reflection at the surface, using the figures given by Sverdrup (1942, p. 53). No allowance has been made for the varying atmospheric absorption with zenith distance, for degree of cloudiness of the sky,

or for sea state. The first of these was disregarded since it varies geographically and seasonally (other than by reason of latitude alone) as well as with atmospheric conditions on a given day. Data given by Haurwitz (1934) indicates that under average conditions, the error caused by this effect will not exceed 20 per cent at zenith distances of less than 60–70°, and 50 per cent at about 80°. Haurwitz (1946) gives figures showing that, with overhead sunlight and ten tenths cloud of maximum density (4), insolation is reduced to 18 per cent. At an air mass of 5.0 (= zenith distance of 78.5°) the figure is 23 per cent. Poole (1936) states that surface loss is unlikely to be increased by waves and introduced bubbles by more than 10–15 per cent. Since our iso-illumination curves are drawn one hundred fold apart, a reduction of surface light of as much as 90 per cent would only mean an error of a half interval in their depth, so it is felt that the above three sources of error may safely be neglected.

Allowing for the angle of refraction of the light entering the water, we have assumed that this angle will be maintained down to the depth in question, and calculated the illumination from the known coefficient of extinction. This will not hold true if a large proportion of the surface illumination comes diffusely from the whole sky rather than directly from the sun, if directional effect is largely lost at the surface because of wave action, or if there is considerable scattering in the water. Kimball (1924) gives mean figures for the ratio of direct solar radiation to that from the sky. For Washington, D. C., these vary from 5.3 : 1 at zenith distances of 25° or less, to 4.1 : 1 at 60°, and 1.6 : 1 at 78.7°. The major fraction of the incident illumination is, then, directional, except at very low sun altitudes; even of the fraction which comes from the sky, that from near the sun is by far the brightest, so there is still a considerable directional quality in it. Whitney (1941) has calculated the mean path length of a ray for different zenith distances and different percentages of sky radiation. At a zenith distance of 60° and average cloud, sky radiation would be 19.5 per cent of the total. From Whitney's values, this would mean that our calculated ray path would be 1.9 per cent too long, which is a negligible error for the present purposes. The error would not exceed 10 per cent at a zenith distance of less than about 80°. Since surface loss by reflection is only 6 per cent at a zenith distance of 60°, and 25 per cent at 80°, the variation in this with cloud or sea state may be neglected. Waves will, in general, tend to decrease the obliquity of the ray and hence its ray path. No data are available on the magnitude of this effect. An estimate of the effect was obtained as follows. The Stokes and Mitchell wave given by Sverdrup *et al.* (1942, p. 527) may be taken as an approximation of the steepest average condition which will be met with. A series of ten parallel rays was considered, spaced equally along a horizontal surface from crest to crest of this wave. The path of each after refraction was calculated, and the mean ray path obtained. Comparison of this, for different solar zenith distances, with ray path for a plane sea surface gave the following results. At Z.D. = 0°, the plane surface path is 1.8 per cent too short, and at 25°, 50°, and 75° it is, respectively, 4.3 per cent, 11.2 per cent and 13.2 per cent too long. Since these figures are based on extreme conditions, it seems justifiable to disregard the effect of sea state on ray path.

Except within about an hour of sunrise and sunset, all the above sources of error may be disregarded. This paper is mainly concerned with illumination at, or near, noon. For detailed study of the periods when the scattering layer is

ascending or descending, allowance will have to be made for certain errors. The most serious source of possible error lies in our assumption that a ray, entering the water at a given angle, will maintain this angle to several hundred fathoms. Various workers (*e.g.*, Whitney, 1941), have pointed out that, if there is any scattering in the water, since vertical rays will have a shorter path downwards than oblique ones, there will, therefore, be a progressive removal of all but the vertical ones. Thus, whatever the incident angle at the surface, below a certain depth all light will tend towards vertical, and an angular distribution plot of illumination will be symmetrical about a vertical axis, and with considerably lower intensities away from the vertical. Such an angular shift with depth has been demonstrated in fresh water lakes, and in some marine localities, but never in water with as low an extinction coefficient as that with which we are concerned. For example, in the most transparent water studied by Whitney (1941) there was an angular shift from about 30° at 10 metres to 25° at 40 metres. From the location, 39°34′N., 71°05′W., one would assume that the extinction coefficient was in the neighborhood of 0.10. Very little of our area has a coefficient approaching this, and in some parts it falls below 0.04. Jenkins (1946) gives data on the relative proportions of scattering and absorption in the attenuation of light in sea water. While the two may be of the same order of magnitude in coastal waters, he considers that scattering is negligible in oceanic waters, except, perhaps, in the top hundred metres. Since we are concerned almost exclusively with oceanic water, it seems reasonable to assume that, if any reduction in obliquity of the light takes place, the reduction will be mainly near the surface, and will be of a lower order than that shown by Whitney (1941, Fig. 5). Until actual measurements are available of the illumination at a depth of several hundred fathoms, we feel that the assumption that there is relatively slight change in obliquity of the rays in the open ocean gives the best approximation to actual conditions. Any error so introduced will be greatest towards sunrise and sunset, and will result in our iso-illumination curves being drawn too close to the surface.

Extinction coefficient data have been collected from all available sources, and charted. There are still so few of these that we feel that inaccuracies in the coefficients which we have made use of are likely to be the most serious source of error in the computation of the illumination curves, particularly as a small difference in extinction coefficient in this very clear water gives rise to a considerable change in illumination at three hundred fathoms. It is unlikely that we are in error by as much as 0.01, except in areas such as the edge of the Gulf Stream, and at the noon depth of the scattering layer this would give rise to an error in the calculated illumination of about 10^{-2}.

The possibility that extinction coefficients decrease considerably with increasing depth in the open ocean is one which requires consideration. It may well necessitate adjustment of our deeper figures. Certain theoretical considerations suggest its probability, but so far any direct measurements are lacking.

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THE RESPIRATORY METABOLISM OF EXCISED TISSUES OF WARM- AND COLD-ADAPTED FISHES

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A striking characteristic of certain arctic poikilotherms such as the arctic (or polar) cod, blackfish, sculpin and indigenous isopods and amphipods is their ability to remain active at environmental temperatures ranging around 0° C. or lower. In the case of the fish there is good reason to believe that body temperature is within a few tenths of a degree of the temperature of the water habitat (Clausen, 1934; Gunn, 1942). Polar cod are often found in sea water in ice pockets and cracks where the water temperature is below 0° C. In contrast, many poikilothermic forms living in the temperate zone are in a state of cold narcosis at such temperature (Parker, 1939). In consonance with these observations is the finding that failure of conduction occurs in the sciatic nerve of the green frog at about 5° C. (Gasser, 1931).

Recently Irving and his associates (Edwards and Irving, 1943a, 1943b; Haugaard and Irving, 1943) described a form of adaptation to temperature in certain non-hibernating poikilotherms. They showed that in these animals the effect of temperature on metabolic rate depends in part on the temperature of the environment from which the animal was taken. Thus, at temperatures below 20° C., the oxygen consumption of the sand crab (*Emerita talpoida*, Say), after allowance for differences in size, was greater in winter than in summer. Both the temperature of maximum oxygen uptake and the thermal death point (range) were lower in winter than in summer (Edwards and Irving, 1943a). This change in the properties of the biological oxidation system on long exposure to cold may be regarded as a primitive form of chemical defense, and the continued growth and activity of the sand crab during the winter indicate that it is an important adjustment of the animal to season. No such seasonal influence on the effect of temperature on oxygen consumption was found in the beach flea (*Talorchestia megalopthalma*), an air-breathing neighbor of the sand crab (Edwards and Irving, 1943b). In line with these differences, the beach flea, instead of remaining active during the winter, went into a state of apparent hibernation beneath the sand.

In the cunner (*Tautologolabrus adspersus*, Walbaum) there was some adaptation of the oxidative metabolism to season, but this was insufficient to allow the necessary physical activity to enable the cunner to remain in the summer habitat during the winter months. This view agrees with the disappearance of the cunner from the shoreline waters in winter (Haugaard and Irving, 1943).

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It has long been established that poikilotherms can be acclimatized to withstand environmental temperatures that are normally lethal (Davenport and Castle, 1896; Vernon, 1899–1900; Loeb and Wasteneys, 1912). The relationship between environmental temperature and lethal temperature has been defined more precisely in a number of acclimatization studies by Hathaway (1927), Fry, Brett and Clawson (1942), Doudoroff (1942, 1945) and Brett (1944). Earlier work has provided other examples of metabolic adaptation to temperature in poikilotherms (Battle, 1926; Britton, 1930; Barcroft, 1934; Fox, 1939) and in hibernating homoiotherms (Tait, 1922; Britton, 1930; Suomalainen, 1939). Wells (1935) demonstrated a variation of respiratory metabolism of certain fish with season, and showed that the oxygen consumption was high in the late winter months and low during the summer months. He concluded that "it seems certain that there is some adaptation to high and low temperatures in fish."

On the basis of the evidence in hand it seems reasonable to assume that the phenomenon of metabolic adaptation to temperature is a rather general one. However, no evidence is presently available as to the nature of this effect. The present work was designed to provide data on the tissue metabolism of cold- and warm-adapted forms in relation to environmental temperature, which might contribute to the elucidation of the marked tolerance for cold shown by arctic cod and provide further information as to the nature of metabolic adaptation to temperature.

Methods

The work dealing with the cold-adapted fish was performed at the Arctic Research Laboratory, Point Barrow, Alaska (Shelesnyak, 1948). The form chosen was the polar cod, *Boreogadus saida*, Lepechin (Jordan and Evermann, 1898; Jordan, 1905, 1923), which is found in large schools around the edges and in crevasses of floating and pack ice. Since the shoreline at Point Barrow is ice-free for only a short time each year, it is necessary to utilize the available time to the fullest. Accordingly, during the period of six weeks when the ice was relatively close to shore, large numbers of fish were caught and maintained in aquaria in the laboratory.[3] While the polar cod is occasionally available at other times by fishing through the ice, the obvious difficulties of extreme cold and ice thickness make procurement very difficult. The water temperature in the habitat of the fish at the time of capture varied from -1.5 to $+2.0°$ C. The fish were maintained in the laboratory in aerated aquaria at temperatures ranging from -1.0 to 0° C.

The experiments with the Golden Orfe, *Idus melanotus* (Heckel and Kner, 1858; Guenther, 1868, 1880; Buiytendijk, 1910), were all carried out in the laboratories of the Department of Physiology at Stanford University. These fish were obtained from a commercial dealer, who maintained the aquarium temperature at 25° C. for a minimum period of one week. Suitable numbers of these fish were transferred periodically to the laboratory aquarium, where they were maintained at 25° C. until used. In general, the fish had been living at a 25° C. environmental temperature for two weeks before use.

[3] We wish to express our appreciation to Professor George MacGinitie, Director, Arctic Research Laboratory, Point Barrow, Alaska, and to his crew of Eskimos for their efforts in obtaining the fish for our use.

The following procedures and methods apply to both species of fish. The fish were decapitated, and the brain and liver were rapidly excised. Since the physical properties of the tissue precluded the preparation of brain slices, the whole brain was finely minced with scissors. Liver slices were prepared by the Lucite template method (Crismon and Field, 1940) by means of a clean dry safety razor blade (Field, 1948). The moist cold box technique was used throughout (Peiss and Field, 1948; Field, 1948). Respiration was measured by the Warburg manometric method (Dixon, 1943; Umbreit, Burris and Stauffer, 1945). The gas phase was oxygen. The center wells of the respirometer flasks contained 10% KOH with Whatman No. 40 filter paper wicks. The liquid phase was Ringer-phosphate, pH 7.4 (glass electrode). Manometric measurements were made in a constant temperature bath, equipped with a refrigerator unit, over a temperature range of 0° to 25° C. At any given temperature, the water bath was maintained at ± 0.02° C. Readings were taken at intervals of 10–20 minutes, depending on the respiratory rate of the tissue at the temperature used, and were carried out for a minimum of 120 minutes. Results are expressed in terms of wet weight Q_{O_2}. Thus Q_{O_2} (wet wt.) denotes microliters of oxygen consumed, measured under standard conditions, per milligram initial wet weight of tissue per hour.

Tissue water content was determined by drying to constant weight at 103° C. The mean water content and range, in per cent, for *B. saida* were: brain, 80.5, range 79.4–82.0, 26 fish; liver, 55.2, range 48.2–70.4, 24 fish. The values for *I. melanotus* were: brain, 78.4, range 76.4–80.4, 18 fish; liver, 74.6, range 71.4–76.8, 14 fish. The large range in *B. saida* liver appears to be due to variable amounts of oil residue in the tissue.

Results

1. Physical measurements

The polar cod was chosen for this study for a number of reasons, chief of which were the relatively high activity of the animals at low temperature, the large number of animals available and the relative ease with which they were obtainable. The Golden Orfe was selected as the warm-adapted fish primarily because it was the most readily available fish, in large numbers, which could withstand the high environmental temperature, and which was similar in body size to the polar cod. While the cod is a marine fish and the Orfe a fresh water form, the sea habitat of the cod we used was one of low salinity, and it has been shown that this species of cod survives readily in fresh water. Table I summarizes the body weight and length data for the two species.

TABLE I

Body weight and length data for polar cod (B. saida) and Golden Orfe (I. melanotus)

	B. saida		*I. melanotus*	
	Weight, gms.	Length, cm.	Weight, gms.	Length, cm.
Mean	21.1	15.5	16.8	13.0
Range	9.8–32.0	10.0–20.5	7.3–25.2	8.5–16.5
Std. dev.	4.7	2.2	3.8	1.9
No. fish	90	90	94	94

For both species of fish, the wet weight of the brain was very similar, averaging about 120 milligrams. There was, however, a wide discrepancy in the size of the liver. In the Orfe, this organ was rather diffuse, and was made up, for the most part, of three long, slender lobes. The average weight for the liver of the Orfe was of the order of 200–300 mg. The liver of the polar cod, in contrast, was large in comparison to the total weight of the whole fish. In most cases it weighed from 750–900 mg., but it was not uncommon to find a liver weighing more than a gram in a fish whose total body weight was on the order of 20 grams.

2. Time course experiments

An extensive series of experiments were made to determine the effect of time on oxygen consumption over the temperature range 0–25° C. In the case of brain and liver tissue of the Orfe, oxygen consumption was a linear function of time at all temperatures for 180–240 minutes, which was the maximum time of observation. The data for the polar cod were more variable with regard to temperature. These data are shown in Table II.

TABLE II

Duration of steady states of oxygen consumption of polar cod brain and liver at graded temperatures. Periods marked with asterisks are those in which oxygen consumption was still constant when readings were terminated

Temperature, °C.	Brain mince	Liver slices
0	300 min.*	360 min.*
5	300 min.*	360 min.*
10	240–270 min.	360 min.*
15	170–210 min.	200–240 min.
20	90–140 min.	120–150 min.
25	10– 50 min.[a]	20– 60 min.[a]

[a] At this temperature there was wide variation in the constancy of oxygen consumption with time. This was more pronounced with the brain mince. See text for further discussion.

It can readily be seen that as the temperature is increased the period of time during which oxygen consumption is constant decreases, this effect being more pronounced with the brain mince. At 25° C. it was possible to obtain satisfactory readings for at least 40 minutes in most preparations of liver slices. However, when brain mince was used at this temperature, many of the runs resulted in die-away curves from zero time. It was possible to obtain 3 readings at 10 minute intervals, during which oxygen consumption was constant, in only about 50 per cent of the runs. Thus, it was considered advisable to represent the oxygen consumption of polar cod brain mince at 25° C. by two figures, one representing the mean value for all determinations on the basis of a one hour period, and the other representing the mean value calculated from those runs in which oxygen consumption was constant for 30 minutes or more. This will be pointed out again when Table III and Figure 1 are discussed.

3. Experiments with polar cod and Golden Orfe brain mince

The oxygen consumption of brain mince from polar cod (*B. saida*) and Golden Orfe (*I. melanotus*) was determined at temperatures ranging from 0°–25° C., at

5° intervals. Approximately 100 fish of each species were used, and from 15–30 determinations were made for a given temperature. These data, together with certain statistical measures, are shown in Table III.

The upper figure at 25° C. for *B. saida* is taken from the portion of the oxygen uptake curves of those determinations that were constant for 30 minutes or more. The lower figure represents the overall mean for all determinations over a period

TABLE III

Respiration of B. saida and I. melanotus brain mince at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.267	.356	.523	.794	1.128	1.649* 1.252
Range	.217– .309	.309– .418	.418– .666	.732– .855	.928– 1.382	.817– 1.513
Standard deviation	.027	.029	.064	.035	.116	.188
Number runs	30	26	30	26	26	18
Number fish	14	21	15	17	15	8

I. melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.078	.114	.137	.203	.361	.602	.926	1.370
Range	.051– .109	.094– .133	.110– .165	.176– .231	.277– .480	.504– .707	.700– 1.181	1.131– 1.702
Standard deviation	.017	.014	.015	.017	.048	.056	.132	.162
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

* This value obtained if first 30 minutes are used for calculating (see text).

of one hour. All other figures are based on readings taken from steady state oxygen uptake. Figure 1 illustrates the data graphically. The upper solid circle at 25° C., is taken from the upper figure in Table III, and the lower solid circle from the lower figure in Table III.

When the logarithm of oxygen consumption, expressed as Q_{O_2}, is plotted against temperature in degrees centigrade, several interesting features are apparent from the curves obtained. Neglecting the 25° C. point for *B. saida,* the cold-adapted fish (solid circles), log oxygen consumption appears to be a rectilinear function

of temperature. The dotted line represents the line of "best fit" as calculated by the method of least squares (Snedecor, 1946). The regression equation is:

$$\text{Log } Q_{O_2} = -0.59 + 0.0318t.$$

The Q_{10}, calculated from this equation, is 2.08.

The curve for *I. melanotus* (open circles) appears to be rectilinear over the range 10–25° C., although it is not possible to decide just where the slope starts to change. The regression line for the rectilinear portion of the curve (dotted line) is:

$$\text{Log } Q_{O_2} = -0.81 + 0.0386t.$$

The Q_{10} calculated therefrom is 2.43.

The most striking aspect of the curves in Figure 1 is the sharp break in the lower curve below 10° C. Such a break indicates an increase in the Q_{10} for the values below 10° C., and does not appear in the curve for the polar cod (Table V). The possible significance of this difference will be discussed later.

4. Experiments with polar cod and Golden Orfe liver slices

The overall data, and certain statistical measures, for the oxygen consumption of liver slices of polar cod and Orfe are shown in Table IV. Figure 2 illustrates the data graphically as with the brain mince experiments. The curves resulting from a plot of log oxygen consumption against temperature reveal the same pattern

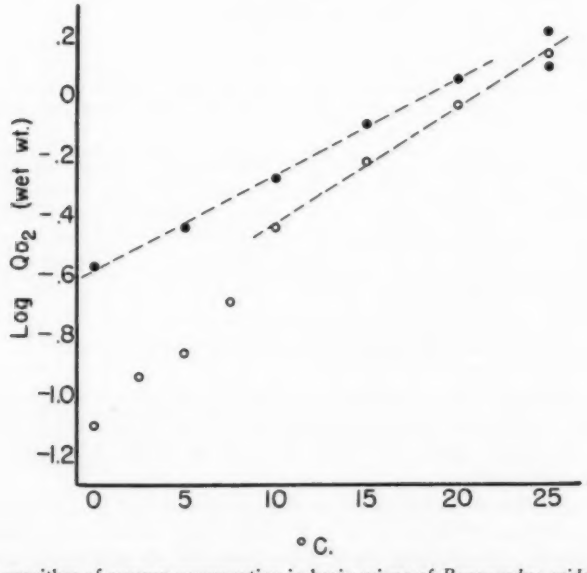

FIGURE 1. Logarithm of oxygen consumption in brain mince of *Boreogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

TABLE IV

Respiration of B. saida and I. melanotus liver slices at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.137	.186	.269	.413	.604	.859
Range	.108–.169	.159–.237	.204–.346	.372–.475	.530–.650	.340–1.093
Standard deviation	.016	.020	.040	.017	.042	.248
Number runs	30	26	34	26	26	18
Number fish	14	21	15	17	15	8

I. Melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.057	.077	.117	.171	.232	.369	.552	.792
Range	.043–.071	.056–.104	.085–.142	.141–.193	.200–.261	.297–.433	.411–.640	.643–.914
Standard deviation	.010	.017	.016	.017	.017	.040	.066	.080
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

as that seen above with brain mince (Fig. 1). The regression equation for the cold-adapted polar cod (solid circles) is:

$$\text{Log } Q_{O_2} = -0.88 + 0.0324t.$$

And the Q_{10} calculated from this equation is 2.11. The regression equation for the warm-adapted Golden Orfe (open circles) is:

$$\text{Log } Q_{O_2} = -0.97 + 0.0352t$$

with a Q_{10} equal to 2.25 over the range 10–25° C. Again, the curve for the cold-adapted form (*B. saida*) appears to be rectilinear over the entire temperature range 0–25° C., while that for the warm-adapted form (*I. melanotus*) is rectilinear only over the range 10–25° C. As in the experiments with brain mince from these two species, the Q_{10} increases sharply for the warm-adapted fish below 10° C. (Table V). Thus, although the respiratory rate of the liver slices is of the same order of magnitude at temperatures of 10° C. and higher, the arctic cod liver respires at progressively higher rate, relative to that of Orfe liver, as the temperature decreases from 10° C. to 0° C. In both brain mince and liver slice, the respiratory rate of

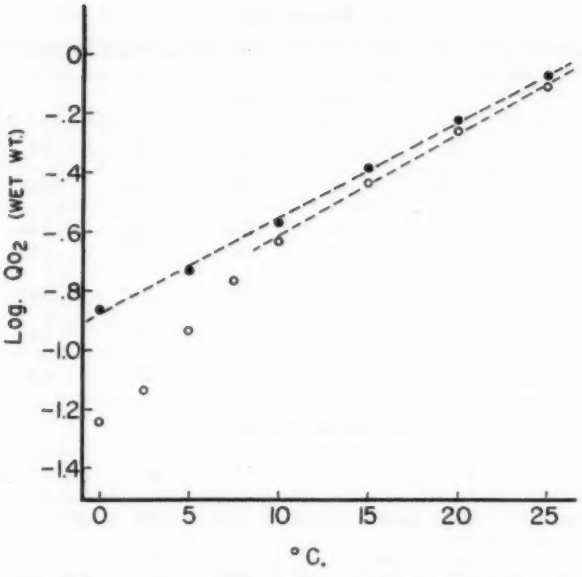

FIGURE 2. Logarithm of oxygen consumption in liver slices of *Boreogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

the tissues from the polar cod is two to three times that of the respective tissues from the Orfe at 0° C. Essentially the two tissues from each species show the same pattern of respiratory metabolism as a function of temperature, but the pattern differs as between species.

TABLE V

Q_{10} values calculated from data in Tables III and IV

Brain mince				Liver slice			
B. saida		*I. melanotus*		*B. saida*		*I. melanotus*	
Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}
0–5	1.78	0–2.5	4.57	0–5	1.84	0–2.5	3.33
5–10	2.16	2.5–5	2.09	5–10	2.09	2.5–5	5.32
10–15	2.30	5–7.5	4.82	10–15	2.36	5–7.5	4.57
15–20	2.02	7.5–10	9.99	15–20	2.14	7.5–10	3.39
20–25	—	10–15	2.78	20–25	2.02	10–15	2.53
		15–20	2.37			15–20	2.24
		20–25	2.19			20–25	2.06
Reg. line		Reg. line		Reg. line		Reg. line	
0–20	2.08	10–25	2.43	0–25	2.11	10–25	2.25

The Q_{10} values calculated from the data in Tables III and IV are given below in Table V, as well as the Q_{10} values obtained from the regression line over that portion of the curve that is rectilinear.

Discussion

The only other work which we may compare directly with our results is that of Fuhrman and co-workers (1944) on the metabolism of excised brain of the large mouthed bass, *Huro salmoides*. Figure 3 is taken from the results of these investigators.

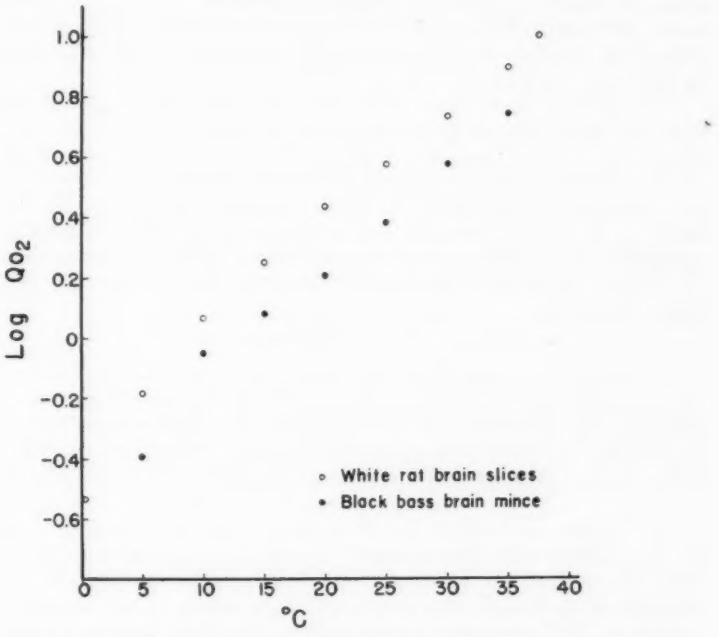

Figure 3. Logarithm of oxygen consumption of white rat brain slices (open circles) and black bass brain mince (solid circles) as a function of temperature.

It is apparent from inspection of this figure that the general pattern is similar to that of *I. melanotus* brain and liver. The Q_{10}'s, respectively, for the rat and bass brain over the range 10–35° C., are 2.14 and 2.06. In each case, over the range 0–10° C., there is an abrupt increase in Q_{10} to the order of 4–5. Thus, the only case we have found where Q_{10} remains essentially constant at the lower temperature is with brain and liver tissue from an arctic-adapted form, the polar cod.

Close inspection of Figures 1 and 2, in which log Q_{O_2} is plotted against temperature, reveals that the log Q_{O_2} may not truly be a rectilinear function of temperature. Other workers have pointed out examples where the rule of van't Hoff does

not adequately express the data. Wells (1935a) measured the respiratory metabolism of Fundulus at graded temperatures and found that the increased oxygen consumption with a rise in temperature was more pronounced at the lower temperatures. He concluded that the data could not be satisfactorily expressed either by the rule of van't Hoff or by the Arrhenius equation. For a discussion of temperature coefficients, see Belehradek (1930, 1935), Crozier (1924), Sizer (1943), Stearn (1949) and Wilson (1949). Similar results were obtained by Ege and Krogh (1915–16) in their studies on oxygen consumption of goldfish. These workers reported a Q_{10} of 9.8 for the range 0–5° C., 3.8 for the range 5–10° C., and over the range 10–28° C. the Q_{10} decreased from 2.9 to 2.2. Both cases, however, appear to follow the general pattern we have seen in tissues of *I. melanotus,* bass and albino rat, as do the results of Gasser (1931, 1933), working on temperature coefficients of spike potential, refractory period and conduction velocity in amphibian nerve.

The present observations show that the arctic cod has at least two advantages over the warm-adapted fish in adaptation to cold. First, at low temperatures the oxygen consumption per unit weight of brain and liver is several times as great; second, the temperature coefficient for oxygen consumption in brain and liver does not rise below 10° C. (as in the Orfe adapted to 25° C. and the bass adapted to 18° C.). Both the high Q_{O_2} and constant rather than increasing temperature coefficient at low temperatures are metabolic features of value in arctic adaptation.

This work was performed under a contract with the Ecology Branch, Office of Naval Research.

Summary

1. Respiratory metabolism has been studied in brain and liver tissues of two species of fish of similar mean body size, the arctic-adapted polar cod (*Boreogadus saida*), living at environmental temperatures of − 1.5 to + 2.0° C., and the Golden Orfe (*Idus melanotus*), living at an environmental temperature of 25° C. Experiments were carried out in the Warburg apparatus over the range 0–25° C.

2. Oxygen consumption in tissues of the Orfe was constant at all temperature levels studied for 180–240 minutes. In tissues of the polar cod, oxygen consumption was constant for 5–6 hours in the temperature range 0–5° C., and then the duration of the steady state decreased progressively as the temperature increased, so that at 25° C. constant oxygen consumption was observed in most cases for no longer than 40 minutes.

3. The Q_{10} for the steady state respiration of tissues of these two species of fish is of the same order of magnitude; values obtained were 2.08 and 2.11, respectively, for polar cod brain and liver, and 2.43 and 2.25 for Golden Orfe brain and liver. However, the Q_{10} remains essentially constant in the case of tissues of polar cod over the entire temperature range studied, whereas it increases sharply in Orfe tissues in the range 0–10° C. Thus, at low temperatures, the oxygen consumption of tissues of the cod, per unit weight, is several times as great as in the Orfe. These relationships are seen more strikingly when the logarithm of oxygen consumption is plotted against reciprocal of absolute temperature, or when the data are analyzed according to the Arrhenius equation.

4. These findings are discussed in relation to the problem of temperature adaptation in poikilotherms. It is concluded that the differences in response of tissues from the polar cod, compared with those of warm-adapted fish, are advantageous in adaptation of the organism to cold.

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BODY INSULATION OF SOME ARCTIC AND TROPICAL MAMMALS AND BIRDS *

P. F. SCHOLANDER,[1] VLADIMIR WALTERS,[2] RAYMOND HOCK,[3] AND LAURENCE IRVING [4]

Introduction

Since the early days of polar exploration it has been known that the arctic in the winter months supports a substantial population of mammals and birds and that these maintain a body temperature that falls within the normal limits found in temperate and tropical forms (Back, 1836; Parry and Lyon, 1825, 1827). All of these warm blooded animals, which include man, may at times be subjected to very low environmental temperatures. The coldest on record are − 68° C. in eastern Siberia and − 65° C. in northwestern Canada, with monthly averages running as low as − 60° C. and below − 50° C., respectively (Bartholomew and Herbertson, 1899; Court, Sissenwine and Mitchell, 1949).

This means for some animals the maintenance, for weeks, of a temperature gradient which may be as much as 100° between the interior of the body and the environment. Thermally this difference is equivalent to maintaining the animals at the boiling point of water in a zero degree environment. Both animals and man accomplish this by two principal adjustments: (1) by lowering the heat loss through increasing the insulation, and (2) by increasing the heat production through raising the metabolism. Actually the smaller mammals and birds, together with man, cannot endure the coldest weather continuously but escape, at least for their resting periods, by burrowing into the snow (ptarmigans), or into well insulated nests where they may stay close together (lemmings, mice, etc.), while others may retire under an electric blanket in a well heated hut (explorers). Finally, the hibernators (arctic ground squirrels) spend the winter within insulated burrows at a very low metabolic rate, just sufficient to maintain body temperature above freezing.

In the winters of 1947–48 and 1948–49, a number of species of arctic mammals and birds were captured near the laboratory at Point Barrow (lat. 71° N.), and were kept in outdoor cages in order to permit study of their means of adjustment to cold. At the same time a collection of winter furs was made.

It was considered that much might be gained in understanding adjustment to cold if we could extend our investigation to include tropical mammals and birds. Through the courtesy of the Navy it was possible to undertake such an investigation during three months in Panama at the U. S. Naval Air Station, Coco Solo, Canal

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Zone, and three months at the Canal Zone Biological Area (Barro Colorado Island), at latitude 9° N.

In the present paper we shall deal with observations on factors that influence the heat loss from the animal, especially the insulation afforded by the fur and feather covering, as measured in pieces of raw skin and fur taken directly from the skinned animal. The arctic furs were all in prime winter condition. Naturally such measurements can only give a rough estimate of how the body insulation varies from one species to another. The living animal may vary the fur and skin insulation greatly by erecting the hairs, by vasomotor control of the skin and tissue temperature immediately under the fur, by changes in evaporation, etc. There are also considerable regional differences in heat loss of body, legs, and face. Respiration and posture of the animal likewise are major factors in determining the total heat loss. Even the relative figures obtained from such skin patch measurements could be misleading as to the total insulation of the animal. We could easily imagine a chubby arctic animal with short, well insulated legs and tail to have a better overall insulation than a tropical animal with long, poorly insulated arms, legs, and tail, even though the tropical form might have a better body insulation than the arctic form.

In spite of these limitations, it was considered that representative insulation measurements of the furs from a series of mammals from the arctic and the tropics would be indispensable for an analysis of the temperature sensitivity in the living species.

The importance of the natural fur and feather covering as an insulator has been long appreciated (Bergmann, 1847; Rubner, 1924; Lefevre, 1911; Tigerstedt, 1910), but few quantitative data on the subject (Giaja, 1931; Babenyscheff, 1938) have been found.

Material

Raw skins were secured from the following arctic mammals, all caught in the winter except the shrew:

Shrew (*Sorex tundrensis*)
Grizzly bear (*Ursus* sp.)
Polar bear (*Thalarctos maritimus*)
Marten (*Martes americana*)
Least weasel (*Mustela rixosa*)
Red fox (*Vulpes fulva alascensis*)
White fox (*Alopex lagopus*)
Eskimo dog (*Canis familiaris*)
Dall sheep (*Ovis dalli*)
Wolf (*Canis lupus*)
Seal (*Phoca hispida*)
Ground squirrel (*Citellus parryi*)
Beaver (*Castor canadensis*)
Lemming (*Dicrostonyx groenlandicus rubricatus*)
Lemming (*Lemmus trimucronatus*)
Hare (*Lepus americanus*)
Reindeer (*Rangifer*—domesticated)
Caribou (*Rangifer arcticus*)

Fat and pieces of musculature were removed from the raw hide and the hair side was carefully dried. The flesh side was left naturally moist for the measurements. Except for a thermally insignificant localized fat pad on the rump of the reindeer and caribou, none of the mammals (except the seals) has any significant layer of subcutaneous fat or blubber. Subcutaneous fat is a heavy and poor insulator compared to fur and does not seem to play any role at all in the insulation of terrestrial arctic mammals. In the aquatic seals and whales, however, it is the principal or

only insulating material. In the seals the skin with blubber adhering to it was detached from the underlying muscle fascia.

Skins from the following tropical mammals were secured in the Panama Canal Zone:

Opossum (*Didelphis marsupialis*)
Night monkey (*Aotus trivirgatus*)
White-faced monkey (*Cebus capucinus*)
Marmoset (titi) (*Leontocebus geoffroyi*)
Three-toed sloth (*Bradypus griseus* or *ignavus*)
Two-toed sloth (*Choloepus hoffmanni*)
Rabbit (*Sylvilagus gabbi*)
Squirrel (*Sciurus granatensis* or *variegatoides*)
Jungle rat (*Proechimys semispinosus*)
Paca (*Cuniculus paca*)
Agouti (*Dasyprocta punctata*)
Raccoon (*Procyon cancrivorus* or *lotor*)
Coati (*Nasua narica*)
Kinkajou (*Potos flavus* or *Bassaricyon gabbi*)
Collared peccary (*Tayassu angulatus*)
Deer (*Mazama sartorii*)

Patches of raw hide large enough for insulation measurements were cleaned, dried, and shipped with disinfectant to the Point Barrow laboratory where the flesh side was remoistened before the measurement. The skin patches were later sent to the Smithsonian Institution, where they were kindly identified by Dr. David H. Johnson and Dr. H. W. Setzer.

Insulation Measurement

The heat transmission through the fur was measured by employing a conventional hot plate and guard ring technique. The hot plate was made of two circular aluminum discs with an insulated hot wire spiral between them. A similarly constructed guard ring, as wide as the radius of the plate, surrounded the center plate. The plate and guard ring could be independently heated and their temperatures read by thermocouples attached to the metal surfaces. The heating of the guard ring could be controlled with sufficient accuracy by a variac; the center plate was heated from a storage battery and the energy input was measured by an ammeter and a voltmeter.

Three sizes of such hot plate guard ring units were employed with the hot plate, 6, 3, and 1 dm.^{2} in area, respectively, depending upon the thickness and size of the skin samples to be measured. Covered with pieces of uniform celotex, the three plates gave a heat transfer reading per unit surface within 3 per cent of each other, and within 5.5 per cent of the figure reported for celotex in the *Handbook of Chemistry and Physics*, 29, p. 1822.

For the measurement, two similar skin pieces (usually from either side of the back on the same animal, or from the backs of two smaller animals) were stretched flat by means of thumbtacks over a circular hole in two plywood frames. The hot plate unit, placed in a flat thin cellophane bag, was sandwiched between the moist flesh sides of the skins and the frames were clamped together (Fig. 1).

To make the measurement, the frame with skins and hot plate unit was hung up vertically in a room thermostabilized at 0° C. ± 1°. The plate and guard ring were heated to 37° C. and held there until the energy input had reached a constant minimum, indicating that heat saturation and equilibrium between heat input and heat loss had been reached. A heavy skin usually required two to three hours before

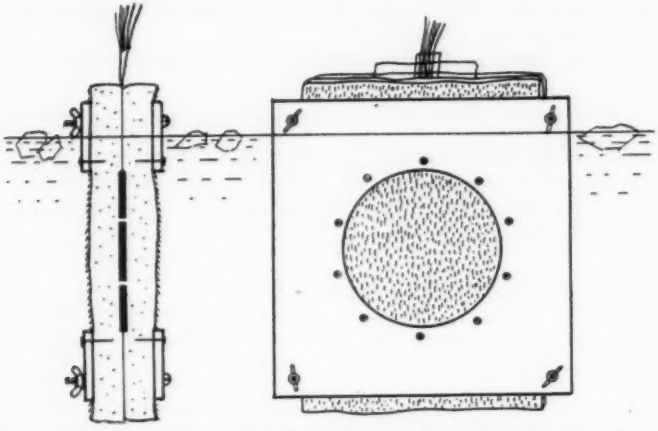

FIGURE 1. Method for measurement of heat transfer through seal skin and blubber in ice water. Left, cross-section through plywood frames holding slabs of seal blubber, clamped around the hot plate guard ring unit. Right, frontal view. Nails are driven through the frame around the center hole to hold the heavy blubber in place. Measurements of the skin of land animals were made with the frame suspended in air at 0° C.

reaching equilibrium. The determinations were made with the fur ruffed up as much as possible for maximum insulating values.

Among arctic animals the insulation of the fur is so large that the hide comprises a relatively small part of the total insulation. The thermal gradients observed in the fur of white fox and caribou showed the surface temperature of the skin to be substantially lower than that of the plate (Fig. 2). This is caused by some ex-

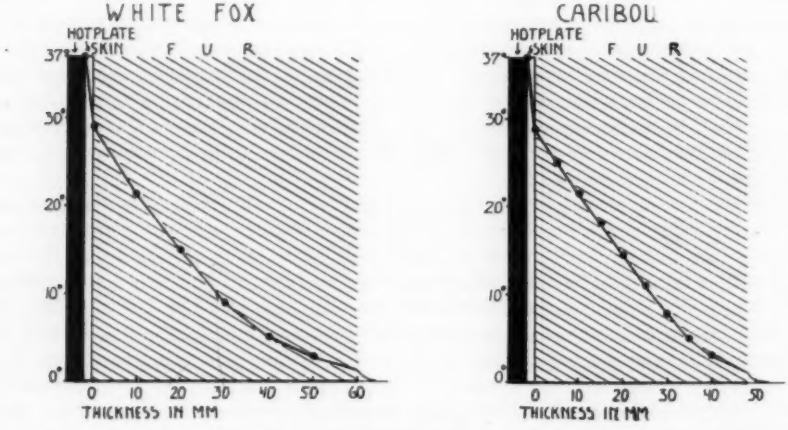

FIGURE 2. Thermal gradients set up in fur of white fox and caribou when in position on the hot plate.

traneous factor, possibly evaporation or poor contact between plate and skin, as it is reasonable to believe that the skin is a poorer insulator than the fur. The decrease in slope of the temperature gradient in the outer layers of the fox fur arises from superficial thinning of the fur. Caribou fur is uniformly dense right to the surface.

In the samples from tropical animals, which often have sparse and short fur, most of the insulation noted in these tests was actually afforded by the hide. This undoubtedly makes the insulation values found for tropical animals considerably higher than in life, when the hide is vascularized, serving heat dissipation rather than heat conservation. In practically naked animals, like the peccary and the paca, the insulation of the fur must be near zero.

Measurement of thickness of the fur is naturally quite arbitrary. A blunt needle was pushed through the fur against the skin and the more or less well defined main surface of the fur set off on the needle with the thumb and measured in millimeters. The average of several such measurements was taken to represent the thickness of the fur.

Insulation in Mammals

The heat transfer was measured in watts per dm.^{2} per 37° C. temperature gradient and the insulation is given as the reciprocal of the heat transfer.[5]

In Figures 3 and 4, observed insulation values are correlated with the thickness of the fur. As was to be expected there is a close correlation with the thickness. None of the furs, under the circumstances tested, were quite as good insulators as an equally thick layer of medium dense cotton. Some of the polar bear furs were conspicuously open and coarse haired and proved to be poor insulators for their thickness.

Among mammals the size of the fox or larger, there is no clear correlation of fur thickness or insulation to the size of the animal (Fig. 5). At the size of the fox, insulation seems to have reached a useful maximum shared by most of the larger forms, including man wearing eskimo parka and pants made of one layer of caribou or other fur. With size decreasing below that of the fox, fur must be shorter and lighter or the animal could not move about. This is particularly true for the smallest forms, the shrew, weasel, and lemming. The ground-squirrels are somewhat out of line, with very little insulation compared to weight. However, they are hardly ever exposed to such gradients as the others, since they escape into their burrows and hibernate. At the lower end of the scale we note that small arctic mammals overlap in insulation value some of the tropical forms, which is a remarkable fact. We shall see, however, that arctic and tropic forms are nevertheless quite different in their temperature sensitivity.

Eskimo dogs and white and red foxes were observed to sleep unprotected on the snow at − 50° C., and so presumably do the wolves, polar bears, caribou, Dall sheep, and other large mammals. The smaller mammals, however, all live in burrows in the winter time and have insulated nests of grass, cotton grass, reindeer

[5] Professor Alan C. Burton has kindly informed us that this unit of insulation amounts to about 2.4 Clo units, and that the slope of the fur insulation in Fig. 3 works out at about 3.7 Clo per inch, which is the accepted value for still air in clothing. An arctic uniform has about 5 Clo units or about 35 mm. thickness. It will be noted that this is considerably less than what most of the larger arctic mammals wear on their body.

hair, etc. Their habits supplement the meager insulation of their fur. As will be shown later, their metabolic heat regulation has already set in before the temperature drops to zero, so that whenever they leave their nests in cold weather they have to be active in order to maintain their body temperature. At Point Barrow the snow temperature at ground level usually stayed at − 25° C. during the winter. One

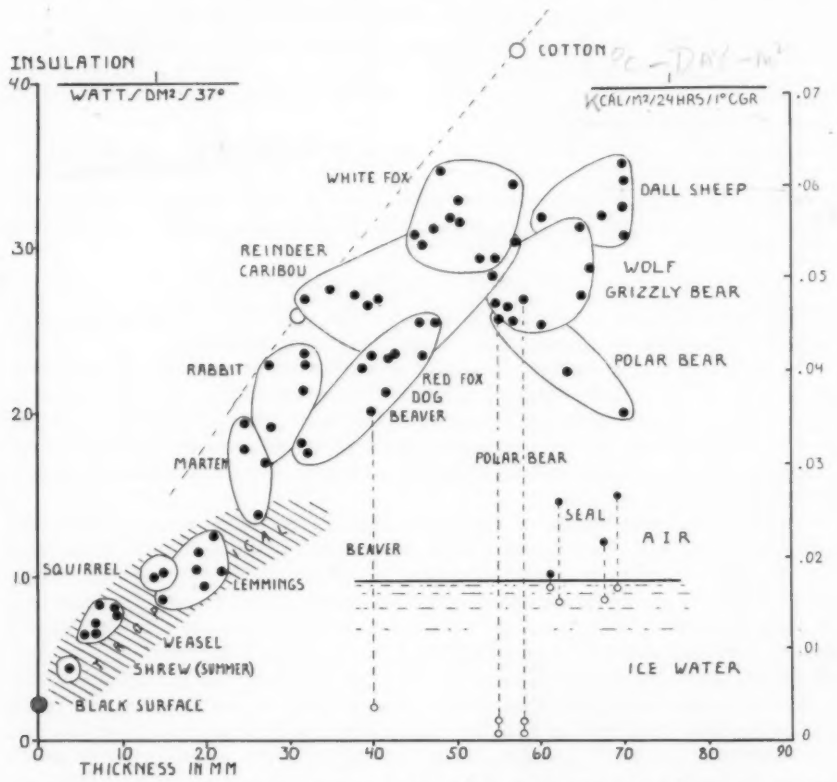

FIGURE 3. Insulation in relation to winter fur thickness in a series of arctic mammals. The insulation in tropical mammals is indicated by the shaded area. In the aquatic mammals (seal, beaver, polar bear) the measurements in 0° C. air are connected by vertical broken lines with the same measurements taken in ice water. In all cases the hot plate guard ring unit was kept at 37° C. and the outside air or water at 0° C. The two upper points of the lemmings are from *Dicrostonyx*, the others from *Lemmus*.

small lemming nest of grass was found in fair condition after the spring thaw and showed an insulating value of the walls, in air, roughly 1.5 times that of the lemming fur. Covered with snow, the insulation could have been no less. Colonial nests, which are much larger, have been observed and it is reasonable to believe that the nests are warm enough to maintain an air temperature around the animals at + 10°

C. or better, which is their critical temperature. Below 10° C. they would have to raise their metabolic rate.

The shrew, weighing only 1.9 gm., was found in the summer time, and its skin was too small to be measured on the smallest hot plate. The insulation of the summer skin has been estimated from its fur thickness. As in the other animals, its insulation is probably greater in winter.

Among the tropical mammals tested (Fig. 4), the sloths are the best insulated, which seems to be necessitated by their low rate of metabolism. The kinkajou and

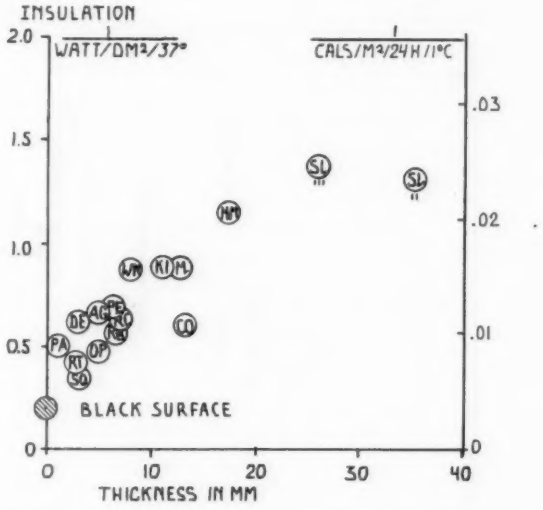

FIGURE 4. Insulation in relation to fur thickness in tropical mammals. Hot plate guard ring unit 37° C., outside air 0° C. The insulation of the fur of most of these animals is probably close to that of a bare black surface but is higher in our measurements because of the relative high insulation of the skin in our dead samples.

AG—agouti	M—marmoset	PE—peccary	SL II—2-toed cloth
CO—coati	NM—night monkey	RB—rabbit	SL III—3-toed sloth
DE—deer	OP—opossum	RC—raccoon	SQ—squirrel
KI—kinkajou	PA—paca	RT—rat	WM—white-faced monkey

night monkey, both active nocturnal and arboreal mammals, are relatively well insulated, whereas other nocturnal ground mammals like the paca, opossum, and jungle rat are among the poorest insulated. There does not appear to be any correlation between insulation and diurnal or nocturnal habits. If anything, it would seem that a night sleeper, passive when it is coldest, might need more insulation than a day sleeper. But the microclimates selected are not known. It should not be forgotten that in many boreal, as well as tropical climates, almost naked mammals live side by side with relatively furry mammals, pointing to the potency of peripheral vasomotor control for the regulation of the body insulation.

Among the smaller arctic mammals like the lemming, weasel, and snow-shoe rabbit, the legs and feet have a considerable fur protection as compared with their southern relatives. However, among the birds that will withstand cold winters, naked legs are most common, and legs insulated with feathers, as in the ptarmigan and snowy owl, are exceptions. In general the insulation on the legs and feet in mammals and birds is very much less than on the body. Several measurements on mountain sheep, caribou, and reindeer showed only from $\frac{1}{5}$ to $\frac{1}{3}$ as much insulation

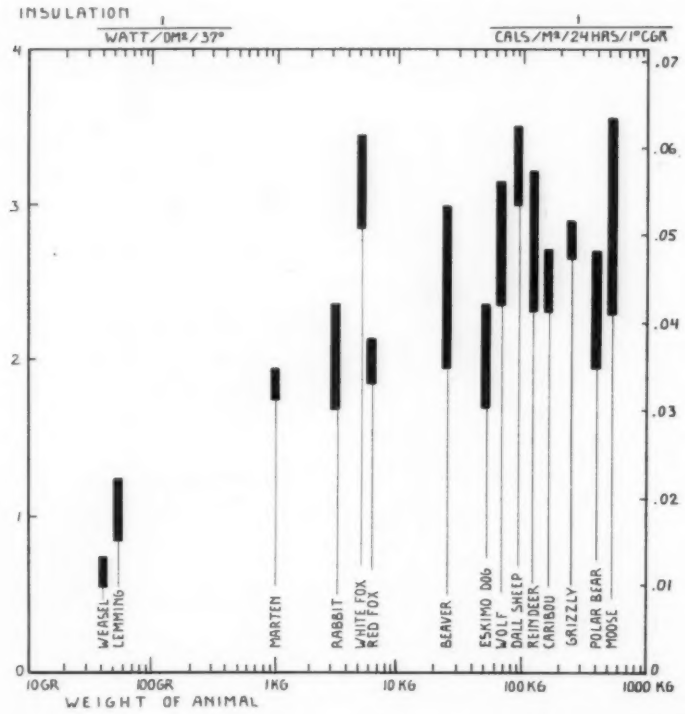

FIGURE 5. Insulation of the winter fur in relation to the body weight in a series of arctic mammals. From a 5 kg. fox to a 500 kg. moose the insulation does not vary much in most of the animals.

on the legs as on the body. We know that aquatic birds can stand low leg temperatures. Undoubtedly seals, muskrats, beavers, moose, and other semi-aquatic mammals can also stand low leg temperatures for a long time, as they virtually lack insulation against the ice water. It seems very probable that arctic nonaquatic mammals and birds can also stand prolonged low leg temperatures because the leg insulation is so slight. This assumption is strongly supported by measurements of the melting point of fats taken from caribou legs. Thus Abrahamsen (1950) found that fats

taken from the lower parts of the legs had a melting point thirty degrees lower than that of fats from the upper parts of the legs. If poorly circulated the legs would cool and further heat loss would be retarded. Fully circulated, the legs would become warm and would greatly facilitate heat loss on account of their poor insulation. This may, in part, explain the fact that a resting, heavily insulated arctic mammal or bird is nevertheless able to change its heat dissipation by a factor of eleven times. By pilo motor reflex alone it seems doubtful whether a caribou or fox could even double its insulation. The fur of a lemming fluffed up to the maximum, provided roughly only twice as much insulation as the fur with the hairs smoothed down.

Insulation in Birds

Insulation measurements were made on snow bunting and ptarmigan skins. The snow buntings gave figures comparable to those of the lemmings and the ptarmigan figures were comparable to those of the marten. However, these measurements are less satisfactory since it is impossible to produce on the test plate the well-ordered elevation of the feathers which the live bird can achieve to produce maximum insulation.

Our snow buntings spent the winter standing on the board floor in their outdoor wire cage with temperatures often as low as $-40°$ C. Only in the coldest weather did these birds, ravens and gulls, find it necessary to protect their naked feet by sitting on them. Only a few of the buntings showed evidence of frostbite on their slender naked feet. A dozen arctic gulls walked about all winter on the snow in an outdoor wire cage with temperatures sometimes as low as $-50°$ C. without freezing their large-surfaced naked webbed feet. A few determinations of the heat dissipation from a live gull's feet in ice water showed it to be very low. It corresponded to only a few cc. of blood an hour, indicating that the temperature of the foot and leg must have been close to zero. Such a low leg temperature can evidently be tolerated for hours at a time and results in general heat conservation for the bird. None of our gulls froze their feet. The circulation must have increased with the increasing gradient so as to keep the feet and legs at just above freezing; and it is likely that the heat loss from the legs and feet, below freezing, increases linearly with the gradient. A gull that had been kept indoors for several months, in a cage usually warmer than 20° C., was observed to freeze the web on its feet, white and hard, in less than one minute after it escaped through an open door onto the snow outside at $-20°$ C. The outer web and parts of some toes subsequently became gangrenous and were lost. Evidently it had lost its cold adaptation or its vaso motor response was not quick enough.

Insulation Measurements in Aquatic Mammals

In man even a few minutes of partial or total immersion in ice water causes unbearable chill and pain, and yet some arctic mammals spend all or parts of their lives swimming about in icy, subzero waters. It is, therefore, of interest to know something about their body insulation in ice water as compared with that in air. Insulation measurements in ice water were made on skins from the polar bear, beaver, and seal (*Phoca hispida*). This species of seal spends most of its life in waters around 0° C. and possesses only a thin hair covering, but it has a thick layer of blubber

(Fig. 6). The blubber is only sparsely vascularized. When the seal submerges the water penetrates the fur completely, leaving no insulating air layer between the skin and the water.

In a series of experiments the heat transmission through the skin and blubber, when submerged in ice water, was measured (Fig. 3). Similarly, the heat losses in ice water through polar bear skin and beaver skin were measured. It can be seen that seal fur and blubber is not a good insulator in air, only slightly better than

FIGURE 6. Cross sections of two frozen seals (*Phoca hispida*) shot in March, 1948, showing the thick layer of blubber. Tape measure in inches.

that of a lemming, although more than three times as thick. The effectiveness of blubber as an insulator in water is demonstrated by the fact that in one of the seal specimens tested, the insulation in ice water was only 5 per cent less than in zero degree air, which means that the temperature gradient in air and in ice water was taken up almost completely within the blubber, leaving the skin surface correspondingly cool in either medium. In all observations the insulation in ice water was about the same, and the difference from the insulation in air can most likely be ascribed to differences in the fur cover. In a living seal the insulation of the skin

and blubber is undoubtedly even less than in our samples because of some subcutaneous and cutaneous circulation. It must be remembered, however, that as long as the seal is in water the temperature gradient is always moderate, never over 40°. Furthermore, the seal is a bulky animal with a high metabolic rate (Irving, Solandt, Solandt and Fisher, 1935; Scholander, 1940). It has in its flippers a highly effective vasomotor control (Scholander, 1940; Irving, Scholander and Grinnell, 1941), and it can readily be seen how even a slight increase in the peripheral circulation could remove large quantities of heat generated by swimming.

In contrast to the seal, the heat loss through polar bear skin increases 20–25 times when submerged in quiet ice water, and 45–50 times when the water is agitated. The ice water penetrates immediately to the skin surface, dislodging all air, and there is no subcutaneous blubber to afford insulation. Undoubtedly, therefore, the polar bear upon immersion suffers a considerable heat loss at the skin surface. This is probably reduced by peripheral vasoconstriction and cooling and can apparently be compensated for over a long time by heat production during swimming. The coarse and open polar bear fur sheds water very easily upon shaking, so that the insulation is readily restored when the bear emerges. The polar bear is also a very large animal with large heat capacity and has a proportionately small surface.

In contrast to the seal and polar bear, the beaver has an extraordinarily dense and fine fur which retains a layer of air several millimeters thick next to the skin when submerged. This can be easily observed on a submerged skin sample. It undoubtedly helps the insulation, which nevertheless is surprisingly low in ice water.

Summary

Insulation measurements on raw skins from 16 arctic and 16 tropical mammals are given. There is, as would be expected, a good correlation between the thickness of the fur and the insulation. The smaller arctic mammals (weasels, lemmings) have much less insulation than the larger and overlap many of the tropical forms. From the size of a fox to the size of a moose there is no correlation between insulation and body size, they all have about the same insulation per surface area. When submerged in ice water, seal blubber retains about the same good insulation, as compared with measurements taken in 0° C. air. In the polar bear, heat transfer through the fur increases 25–50 times when submerged, because of complete wetting of the skin surface and absence of blubber. The beaver is slightly better off when submerged, as it retains an insulating layer of air in the fur next to the skin.

Acknowledgments

We are greatly indebted to the officers, civilians, and natives of the Barrow camp for the many efforts that they expended in our behalf. We wish to express our indebtedness to Captain H. W. Taylor, USN, Commanding Officer at the U. S. Naval Air Station, Coco Solo, Canal Zone, for facilitating our work there in every way. We also wish to acknowledge with gratitude the great help given us by Dr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone. We are much indebted to Dr. David H. Johnson and Dr. H. W. Setzer at the Smithsonian Institution for kindly identifying the scant skin fragments of the tropical animals, and to Mr. Robert H. Stapleton and Mr. Walter B. Flagg for assistance in many ways. Dr. R. Wennesland rendered valuable assistance in the insulation measurements of arctic furs. We are greatly indebted to Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton for inspiring discussions and criticism.

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HEAT REGULATION IN SOME ARCTIC AND TROPICAL MAMMALS AND BIRDS *

P. F. SCHOLANDER,[1] RAYMOND HOCK,[2] VLADIMIR WALTERS,[3] FRED JOHNSON,[4] AND LAURENCE IRVING[5]

In the preceding paper are presented observations and measurements of the body insulation of a series of arctic and tropical mammals and birds. In the present paper we shall describe experiments in which our animals were subjected to gradually lowered air temperatures in a respiration chamber, where the heat production was simultaneously determined through the oxygen consumption or carbon dioxide production. Similar experiments have been conducted earlier on many laboratory mammals and birds and a few wild animals. Excellent data were obtained by Theodor (1878), Falloise (1900), Rubner (1902), Martin (1902), Goto (1923), Terroine and Trautmann (1927), Benedict and MacLeod (1929), Giaja (1931), Kayser (1930, 1937), Benedict and Fox (1933), Gelineo (1934), Swift and Forbes (1939), Kendeigh (1939, 1944), Kalabukhov (1940), and Herrington (1940).

From the earliest investigations it was made clear that down to a certain environmental temperature (the critical temperature) the body temperature could be maintained, without increasing the metabolism, simply by increasing the insulation (so-called physical heat regulation). Below the critical temperature the body temperature could be maintained only by increasing the heat production (so-called metabolic or "chemical" heat regulation). For geese and rabbits it was found that the critical temperature could be as low as 0° C., and from observed fact that eskimo dogs sleep on the snow at − 40° C. or below we could expect to find arctic mammals having a critical temperature that low or even lower. It was considered likely that cold experiments on tropical mammals and birds would furnish a valuable contrast to our arctic material, and through the courtesy of the Navy we were granted a six months stay in the Panama Canal Zone for the study of this and other problems.

Animal Material

The following arctic mammals were used in our experiments at Point Barrow, Alaska, latitude 71° N.:

White fox (*Alopex lagopus*)
Eskimo dog (*Canis familiaris*)
Polar bear cubs (*Thalarctos maritimus*)
Ground squirrel (*Citellus parryii*)
Lemming (*Dicrostonyx groenlandicus rubricatus*)
Weasel (*Mustela rixosa*)

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and the following birds:

Arctic gull (*Larus hyperboreus*)
Canada jay (*Perisoreus canadensis*)
Snow bunting (*Plectrophenax nivalis*)

The dogs, foxes, and polar bear cubs were kept in outdoor wire cages at an average temperature of − 25° C. The weasels, lemmings, and ground squirrels were kept in an animal house at around − 10° C. to + 5° C. These small animals all built nests into which they could retire and in which they spent most of their time. The gulls and snow buntings were caught near the laboratory in September and were kept outside in wire cages. Normally they migrate south during the winter. The jays were caught 200 miles south of the laboratory in April. All of the animals outdoors had access to indoor nest boxes through openings in the wall of the animal house; these, however, they seldom used, even at temperatures − 40° C. or below.

The tropical mammals and birds used for our cold experiments were all caught in the Canal Zone (Panama) and were kept in wire cages at the Barro Colorado Island laboratory (latitude 9° N.) where the outdoor temperature is usually 25° C to 30° C.

The following tropical mammals were obtained and used:

Two-toed sloth (*Choloepus hoffmanni*)
Night monkey (*Aotus trivirgatus*)
Marmoset (titi) (*Leontocebus geoffroyi*)
Raccoon (*Procyon cancrivorus*)
Coati (*Nasua narica*)
Jungle rat (*Proechimys semispinosus*)

and the following tropical birds:

Manakin (*Pipra mentalis*)
Night hawk (Paurangue) (*Nyctidromus albicollis*)

Experimental

In order to be able to compare the effect of temperature on the metabolism in widely different mammals and birds we have referred to the average metabolic level at rest as 100, determined as O_2 consumption or CO_2 production in the thermoneutral zone (= temperature zone of lowest metabolism). All changes are given in relation to that figure.

The arctic animals were studied during the winter of 1947–1948 in an interrupted sequence for each animal, in such a way, however, that each animal covered the whole range of temperatures. The basal reference level of heat expenditure could therefore be established empirically for each individual, which eliminated the uncertainty involved in correcting the metabolism for differences in weight. The animals were given a one to two hour period in the chamber at the proper temperature before a series of determinations was made, extending over another period of one to three hours.

The tropical mammals and birds could withstand only a relatively small range of external temperatures and each individual could therefore be run through the whole temperature range in a single day, starting at room temperature and thereafter going down step by step. The animal usually remained in the respiration chamber for eight to twelve hours, with about two hours at each temperature and allowing one hour for bringing the temperature down.

Apparatus

A wooden box was mounted outside one of the windows of the laboratory at Point Barrow. The box was furnished with a window facing the laboratory window and was equipped with a fan and a resistance controlled electric heater. The box could be maintained at any temperature warmer than the outside air. The animal was placed on top of a narrow piece of plywood in a wire screen cage just large enough to hold it without cramping. The cage was placed on a pan furnished with a narrow well containing a non-freezing glycerine solution. Over the cage was put a closely fitting sheet metal respiration chamber resting with its lower edge in the glycerine well to form an airtight seal. The chamber carried tygon tubes for in- and outgoing air and a thermometer. The chamber air was adjusted to the proper

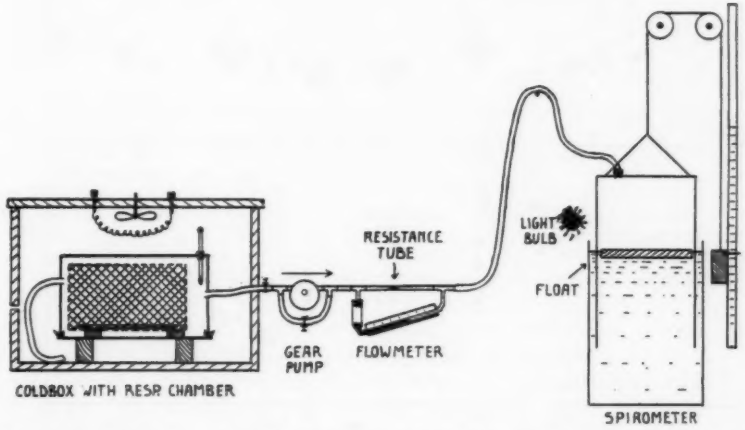


Figure 1. Open circuit metabolism apparatus used in the cold experiments. A wooden float covered with sheet metal protects the water from gas diffusion. The light bulb keeps the spirometer gases well stirred by setting up convection currents.

temperature by regulating the box temperature. It is realized that the air temperature in a small respiration chamber is not an ideal way of characterizing the environmental heat situation. However, it is comparable to all other similar experiments.[6] Heat loss by evaporation was always low because of high relative humidity in the small respiration chamber.

Outdoor air, prewarmed in the wooden box, entered the respiration chamber through a six-foot length of tubing. Air was exhausted from the chamber at the other end by means of a small gear pump which propelled it through a flow meter into a respirometer. The speed of the gear pump and the shunt was adjusted so as to give a ventilation through the chamber which would maintain the carbon dioxide of the exhaust air at about 1 per cent, as checked by gas analysis. As our animals varied greatly in weight and metabolic rates, we needed three sizes of

[6] In his classical work, Martin (1902) gives only the waterbath temperatures. The air temperature in his glass respiration chamber might have been considerably warmer.

spirometers and respiration chambers, two sizes of gear pumps and different bore plastic resistance tubes in the flow meter in order to maintain the same degree of accuracy throughout the series.

Small mammals or birds were usually placed on a narrow piece of plywood in two small tin cans taped together at the openings and furnished with the necessary tubes for in- and outgoing air and temperature measurements. The ventilation was measured on the spirometer and samples were taken out for analysis using either the ½ cc. analyzer (Scholander, 1947) for CO_2 and O_2, or in Panama, the Haldane apparatus for CO_2. A light bulb on one side of the spirometer kept the gases well

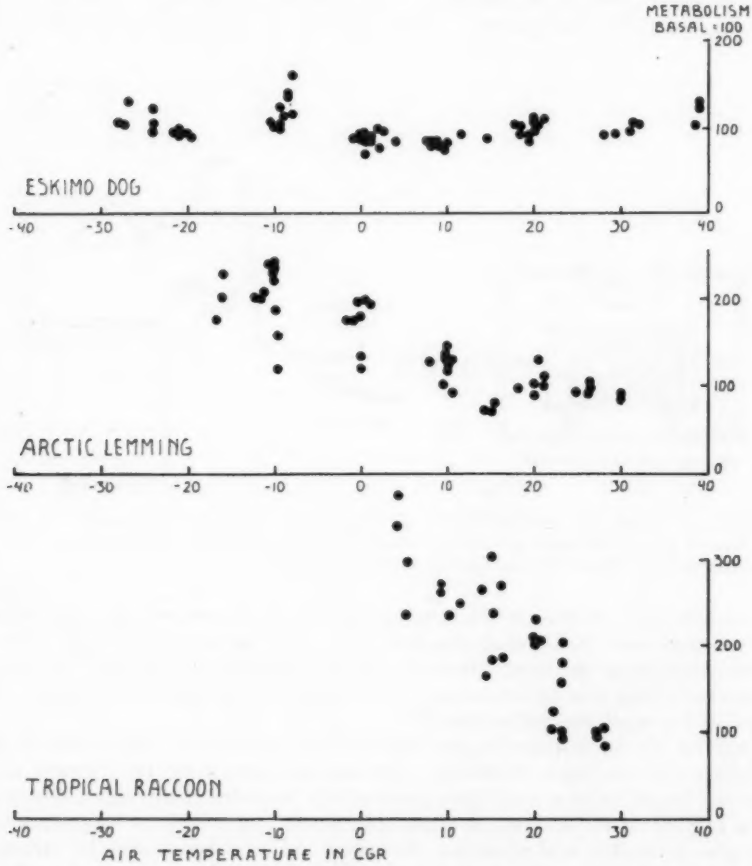


FIGURE 2. Effect of environmental temperature on the metabolic rate in eskimo dog, arctic lemming, and tropical raccoon expressed in terms of basal metabolic rate = 100. The steepness of the curves describes the relative temperature sensitivity of the animal but does not correlate with the weight of the animals or with their body insulation.

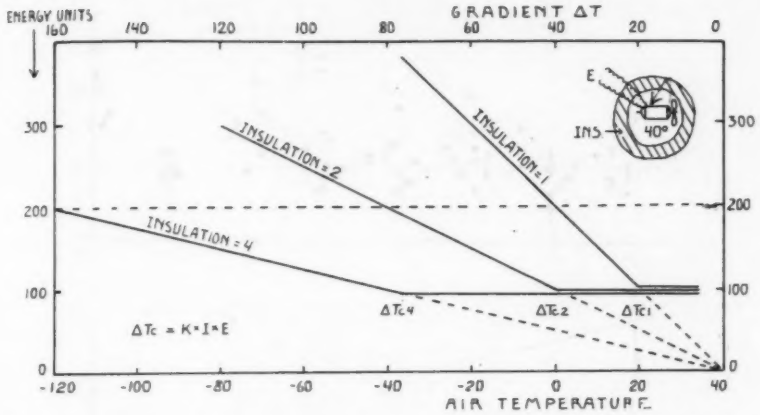


FIGURE 3. Diagram of a thermoregulated system consisting of an electric fan inside a rigid insulator (insert). The full drawn lines represent the relative energy input necessary to maintain the "body temperature" of 40° C. for different air temperatures, assuming a minimum (basal) energy level of 100 and a maximum insulation of 1, 2, and 4 units. The thermoregulation is assumed to proceed in two steps: first an increase in insulation until it reaches maximum at the critical temperature, then an increase in the metabolic rate. At the critical temperature the body-to-air gradient is called the critical gradient ΔTC. At constant insulation the system cools proportionally to the gradient and it will be seen from the figure that the critical gradient is proportional to both insulation and metabolic rate according to the equation given.

stirred by setting up slight convection currents in the spirometer. The accuracy obtained by this well established open circuit technique was ample for our purpose.

For cooling the tropical animals, a deep freeze box was used with an insulated wooden lid furnished with fan and heater. In this was placed the respiration chamber as already described.

The rate of oxygen consumption (arctic animals) or CO_2 production (tropical animals) relative to temperature was plotted for each individual animal, the basal level taken from the plot, and the data recalculated and replotted relative to the basal level which is called 100 (Figs. 2–11).

In the large series of arctic experiments performed by Hock, both CO_2 production and oxygen consumption were determined. The RQ was rarely outside 0.70–0.85 all through the temperature ranges, and hence either oxygen consumption or CO_2 production can be taken as proportional to the heat production, and either one can accurately enough serve our purpose to describe relative changes in heat production.

THEORETICAL CONSIDERATIONS

It became clear from the very beginning of these experiments that the larger arctic mammals like the fox and dog did not need to increase their heat production to stand the relatively modest temperature of − 30° C. that we were able to offer them, and that their metabolism remained essentially unchanged from + 30° C. to − 30° C. However, the smaller arctic mammals and birds soon reacted by slowly increasing their metabolism with falling temperature.

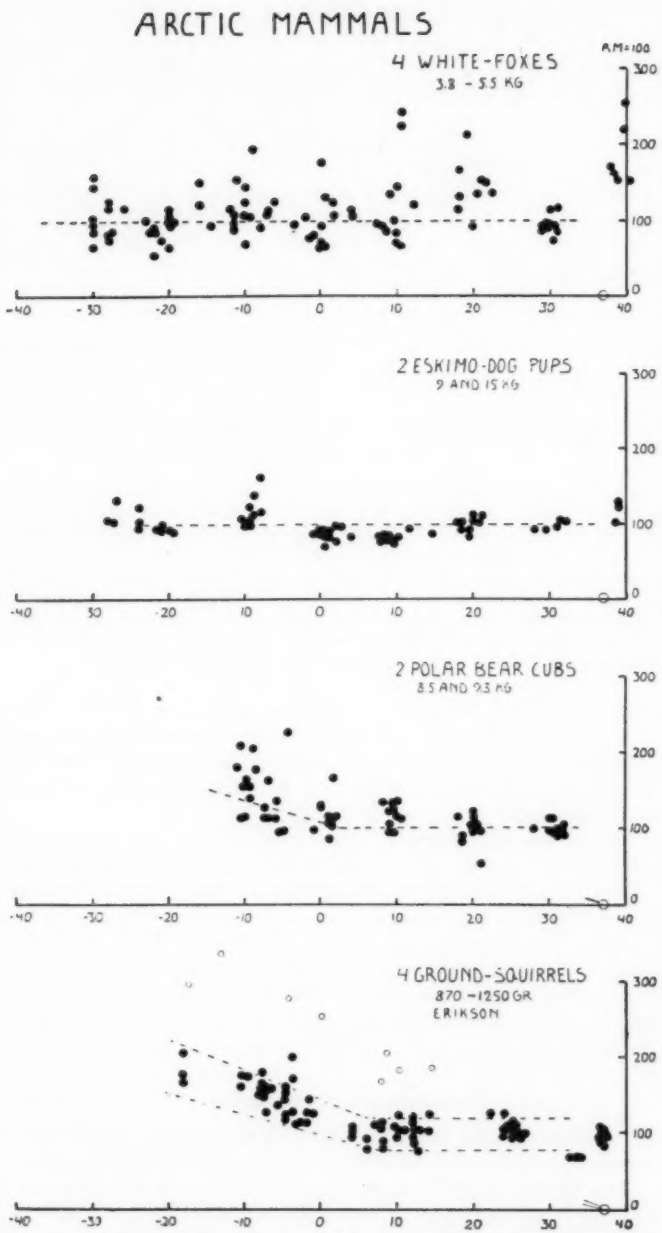


FIGURE 4. Metabolic heat regulation in arctic mammals. In the foxes and husky pups thermoneutrality extends from + 30° C. to at least − 30° C. The open circles in the ground squirrel plot (Erikson, 1950) indicate very active runs where the animal tried to escape.

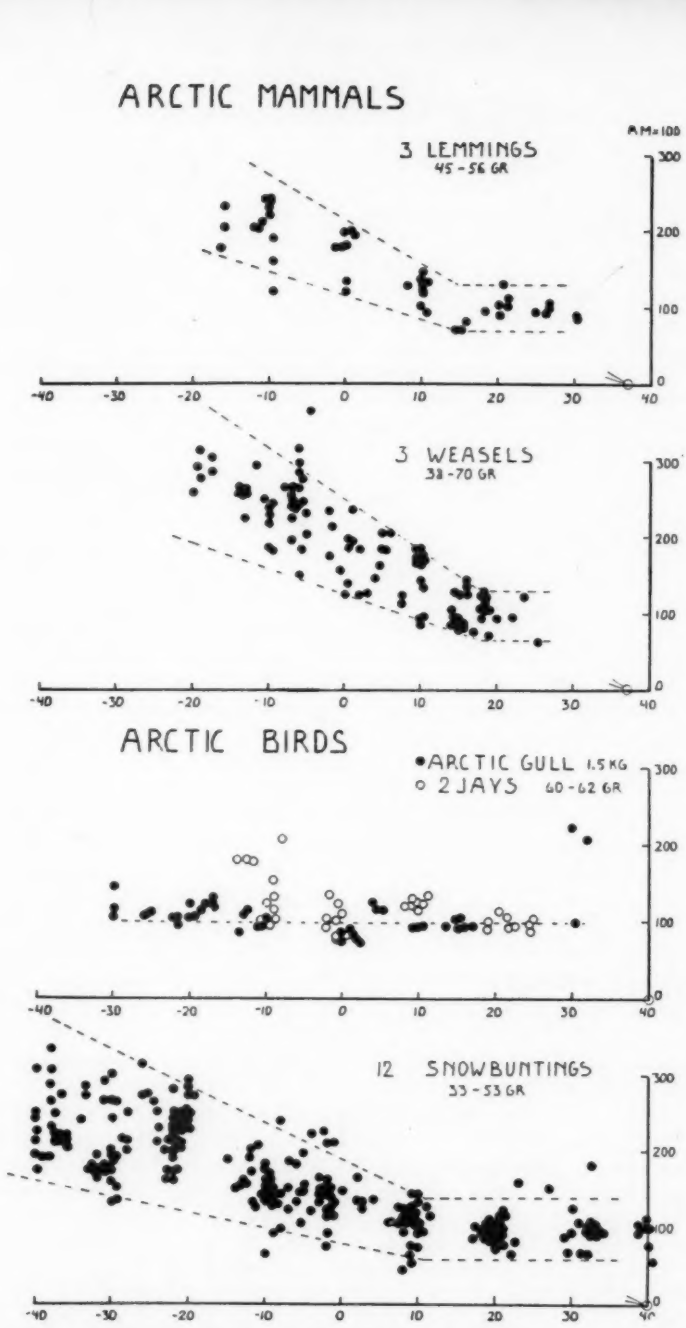
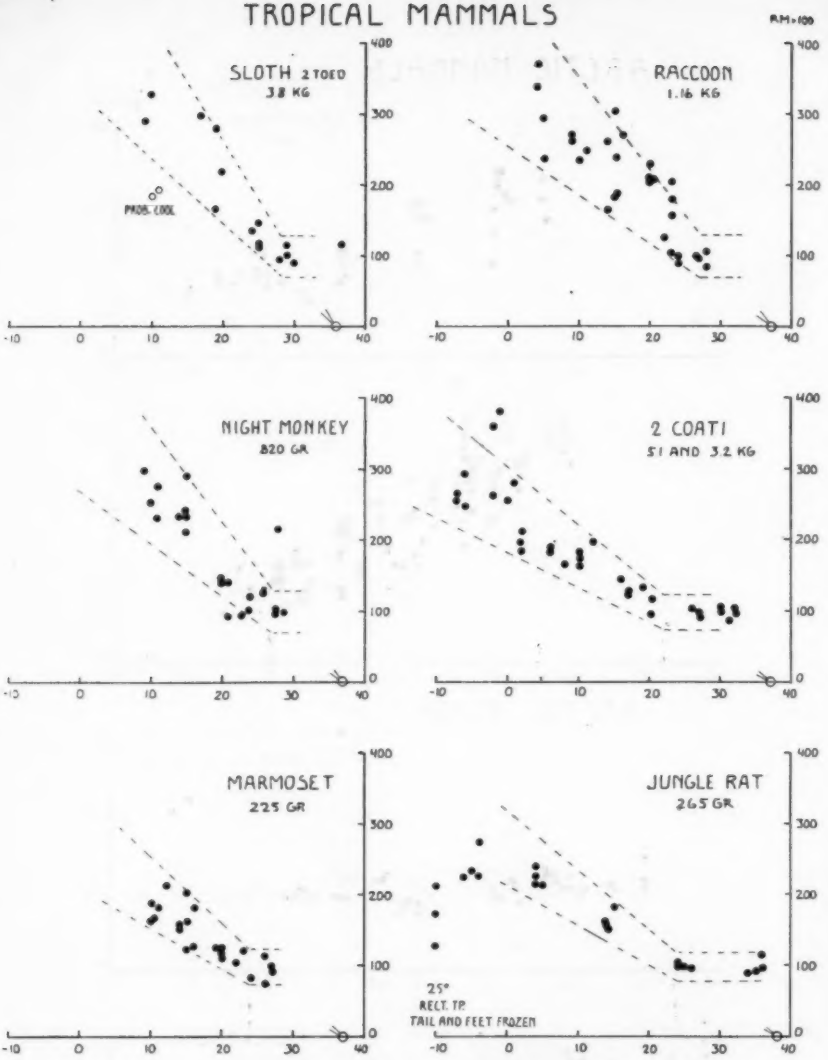
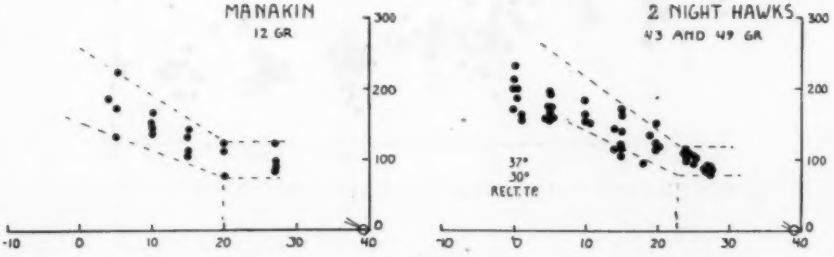


FIGURE 5. Metabolic heat regulation in small arctic mammals and in arctic birds.

TROPICAL MAMMALS



TROPICAL BIRDS



In Panama, it was established that the tropical mammals and birds responded with an increase in metabolism starting at only a few degrees below the ambient air temperature, producing strikingly steep curves compared with those of the arctic animals (Fig. 2). Such differences in slope had been observed by Lapicque (1921) in different animals, and the curves were considered by him to flatten out with increase in size of the animal (Brody, 1945). The relation to size does not hold for the three animals given in Figure 2, however, for the steep-curved tropical raccoon is many times heavier than the little flat-curved arctic lemming. It might be assumed that the curves would become less steep with increasing insulation. However, the tropical raccoon has about the same insulation as the arctic lemming but has a much steeper curve. Altogether contrary to the assumption that insulation alone determines the slope, is the fact that a sloth has a much steeper curve than the less well insulated arctic weasel (Figs. 5 and 6).

Some simple physical considerations lead to an explanation which fits the facts surprisingly well and which also explains, for instance, how an arctic animal can endure temperatures that occasionally may drop as low as $-70°$ C. A physical analogy or model is useful to clarify what seem to be the main points.

Let us consider a warm-blooded animal as a unit capable of maintaining a constant body temperature in a changing environmental temperature, and that it can do this by adjusting (1) its insulation and (2) its metabolism. The major part of the metabolic increase which a mammal or bird can mobilize against external cooling results from muscular movements in the form of shivering and gross activity. We shall, therefore, picture our warm-blooded animal as an electric motor enclosed in an insulated bag. We shall assume a constant surface area and configuration of the bag and we shall maintain the interior of the bag at body temperature, say 40° C., by regulating the energy input to the motor. We are only studying situations of steady-state heat equilibrium where rate of heat loss equals rate of heat production. It has long been known that the overall heat loss of such a system in a uniform air current is proportional to the temperature gradient between the inside and the outside, according to Newton's law of cooling (Mitchell, 1901). That this also holds true in still air with considerable accuracy is evident from experiments by Burton, 1934 (16° gradient), and by Winslow, Gagge, Greenburg, Moriyama and Rodee, 1935 (27° gradient), and from numerous experiments with heated clothing dummies (copper man). According to the radiation law of Stefan (McAdams, 1942) we should expect, with increasing gradients, progressively smaller values than those predicted from Newton's law. However, as the surface of a good insulator (such as the fur in our arctic animals) progressively cools with the environmental temperature, we are mostly dealing with surface to air gradients which hardly exceed 10–20° and the calculated discrepancy for such small gradients is negligible, especially when compared with the great variation found in our animal experiments. In the present connection, therefore, Newton's law of overall cooling of physical

FIGURE 6. Metabolic heat regulation in tropical mammals and birds. In most of them there is a fair proportionality to the body-to-air gradient. At the lowest temperatures the metabolism dropped in the jungle rat and the night hawks, as a result of falling body temperature. The two circles in the sloth plot were the last determinations and the animal was undoubtedly cool (no body temperature taken).

bodies can be applied as a natural basis for discussing the overall heat loss of animated bodies at different temperature gradients.[7]

Our heat regulating model is pictured in Figure 3, showing an electric motor (fan) in an insulated bag and a diagram relating the energy input necessary to maintain a constant body temperature for different gradients and insulations.

For a given insulation value (1) of the system, the energy input for different temperature gradients (ΔT) would follow the diagonal (Ins. = 1), starting with zero energy at zero gradient. If the total insulation were twice as good, the rate of heat trasfer would be only half and the energy input would consequently be only half, as indicated by the diagonal (Ins. = 2). Similarly with four times as good an insulation the rate of energy input would be one quarter as great as one and would be represented by the diagonal (Ins. = 4).

Now let us assume that our model, like the animals, can regulate its insulation and that it has a basal level below which the energy production does not fall. We shall further assume that the thermoregulation in our model always proceeds according to the following sequence when the external temperature falls: first, a gradual increase in the insulation alone until it has reached its maximum and thereafter a gradual increase in the energy input. In an animal the first part would correspond to: a zone of physical heat regulation, which at the critical temperature would change into the second part: the zone of metabolic (chemical) heat regulation, *i.e.*, we presume a complete separation between the physical and chemical heat regulation. Suppose now that we start with our model in air at + 30° C. As we lower the temperature we increase the insulation correspondingly, running horizontally, until we reach 20° C. air temperature. If the insulation is then at its maximum (Ins. = 1) the curve will break and the energy input will increase proportionally to the gradient according to the diagonal marked Ins. = 1. If, however, the insulation can be steadily increased to a value of 2, the critical temperature will be at 0° C. and the energy input will, from then on, follow the line marked Ins. = 2. Similarly if the insulation can be increased 4 times and no more, the critical temperature will move to − 40° C. and from then on the energy input will again be proportional to the gradient and will follow the diagonal marked Ins. = 4.

We have, according to convention, called the lowest temperature at which our model or animal can maintain body temperature without increasing metabolism, its critical temperature (TC), and we shall designate the corresponding temperature gradient between body and environment as the critical gradient (ΔTC). In other words, critical gradient is the greatest temperature gradient which the physical heat regulation alone can take care of.

It will be seen from the Figure that the critical gradient for any given energy level is directly proportional to the insulation. If a system (Ins. = 1) has a critical gradient of 20° C. and is at 20° C. air temperature, doubling the insulation would double the critical gradient and move the critical temperature to 0° C. If another system (Ins. = 2) has its critical temperature at 0° C. and doubles its insulation, the critical gradient will double and the critical temperature move out to − 40° C. Similarly, if a system with a fixed energy level can maintain a constant body tem-

[7] Dr. Alan C. Burton has kindly informed us that the theoretical drop from linearity by the radiation equation in actuality is counteracted by an increase in convection loss due to increased heat capacity as air gets cooler.

perature of 37° C. in an environmental temperature that changes from + 30° C. to − 40° C., as an arctic fox or dog can do, then we know that the gradient increased from 7° C. to 77° C. and hence that the insulation also increased by the same amount, namely, 11 times!

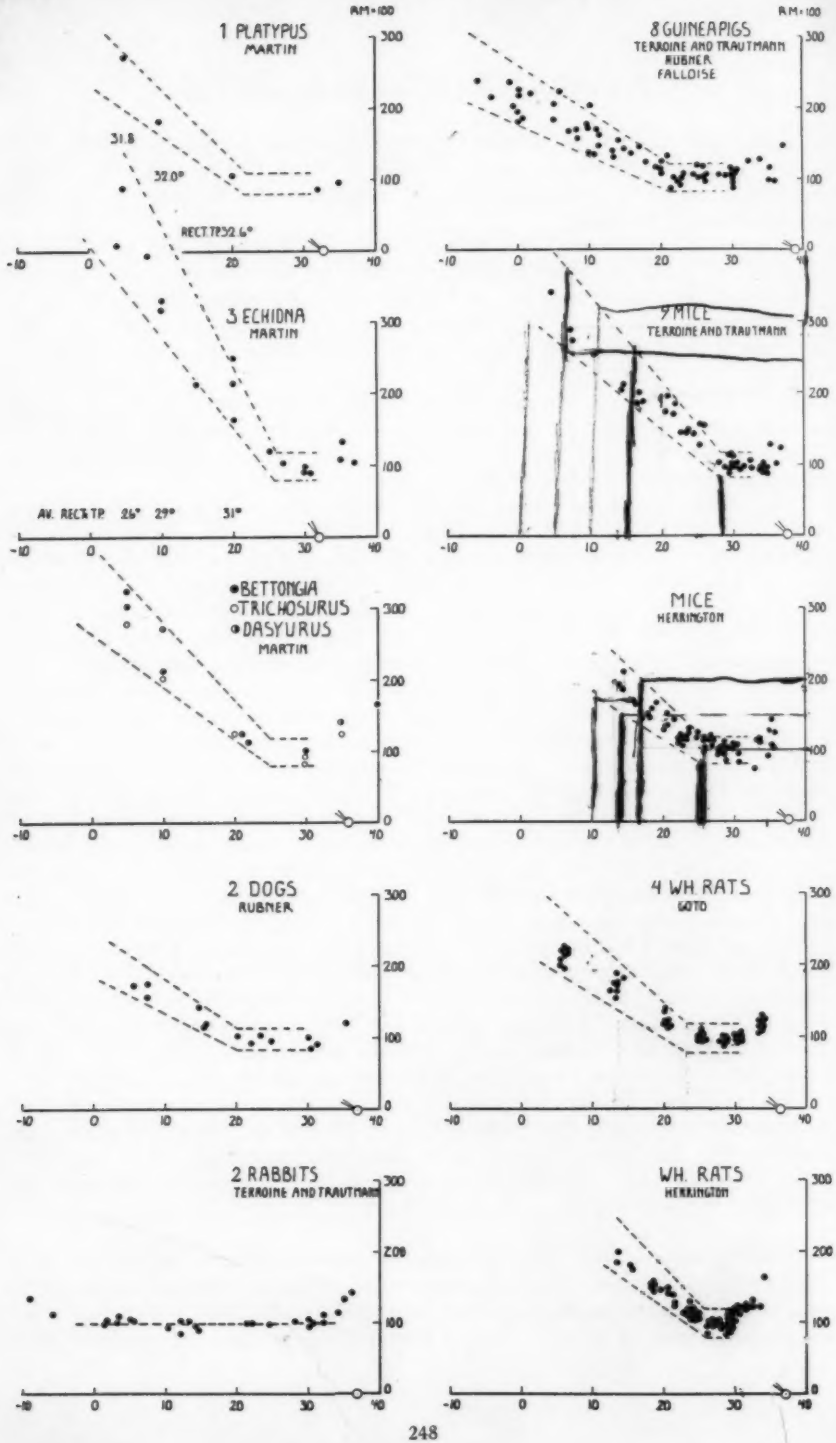
If we now consider the three systems (Ins. = 1, 2, and 4) and we double the energy level in all of them (from 100 to 200) we see that the critical gradient (ΔTC) will also double in each case; in other words, the critical gradient is proportional to the energy level. Physiologically this is of considerable interest. Doubling the metabolism in each system will move the critical temperature of Ins. = 1 from 20° C. to 0° C., of Ins. = 2 from 0° C. to − 40° C., and of Ins. = 4 from − 40° C. to − 120° C.! An arctic fox with body temperature of 40° C. and a critical temperature at − 40° C. can, merely by doubling its metabolism, go to − 120° C., and it takes only a 37 per cent increase in heat production to sustain it in the coldest temperature recorded on earth, somewhere near − 70° C.!

The dependence of the critical gradient on the metabolism and the overall insulation can be combined in a simple formula: stating that the critical gradient is proportional to the product of the basal metabolism and the maximal overall insulation, or $\Delta TC = K \times I \times E$, where ΔTC is the critical gradient, K is a constant denoting the units, I is the maximal overall insulation of the system and E is the basal energy level. As we shall see, this explains why a sloth, with a greater fur insulation than the arctic weasel and lemming and our other tropical animals, is still one of the most temperate sensitive animals because of its well known low metabolism.

We have now discussed some fundamental properties of a simple thermoregulated system with only two variables, insulation and metabolism. Our next task is to see to what extent we can expect this analogy to be applicable to a homoiothermic animal. Most authors (Hoesslin, 1888; Lefevre, 1911; Terroine and Zunz, 1925; DuBois, 1936) have emphasized the observation that heat loss from a warm-blooded animal is not proportional to the temperature gradient, and generally speaking this is true. The matter has been somewhat confused, however, by the failure to realize that proportionality should only hold below the critical temperature. Dahr (1926) cites the case of a guinea pig experiment of Rubner's as evidence that they lost heat according to Stefan's law of radiation, but uses body temperature instead of surface temperature. Actually his example nicely illustrates a linear relation to the body gradient. Martin (1902) points out that the heat production of Echidna is proportional to the gradient. Lapicque (1921) and Kleiber and Dougherty (1934) assume, for theoretical reasons, an approximate applicability of Newton's law of cooling below the critical temperature, but do not furnish any factual evidence for it.

To what extent Newton's law would hold below the critical temperature depends upon several factors, the most important being (1) the constancy of the body temperature, (2) the degree of constancy of the overall insulation below the critical temperature, and (3) the degree of constancy of the heat production for each temperature. We shall discuss these factors below.

1. Body temperature. Generally speaking, the least variable factor in the whole picture of homoiothermy is the body temperature (Wetmore, 1921; Wislocki, 1933). The poorest temperature regulators are bats, which in the resting state are practically poikilothermous (Hock, 1949). Relatively poor regulators are the mono-


AM=100
1 PLATYPUS
MARTIN
31.8
32.0°
RECT.TP32.6°
8 GUINEAPIGS
TERROINE AND TRAUTMANN
RUBNER
FALLOISE
3 ECHIDNA
MARTIN
AV. RECT TP 26° 29° 31°
9 MICE
TERROINE AND TRAUTMANN
BETTONGIA
TRICHOSURUS
DASYURUS
MARTIN
MICE
HERRINGTON
2 DOGS
RUBNER
4 WH. RATS
GOTO
2 RABBITS
TERROINE AND TRAUTMANN
WH. RATS
HERRINGTON

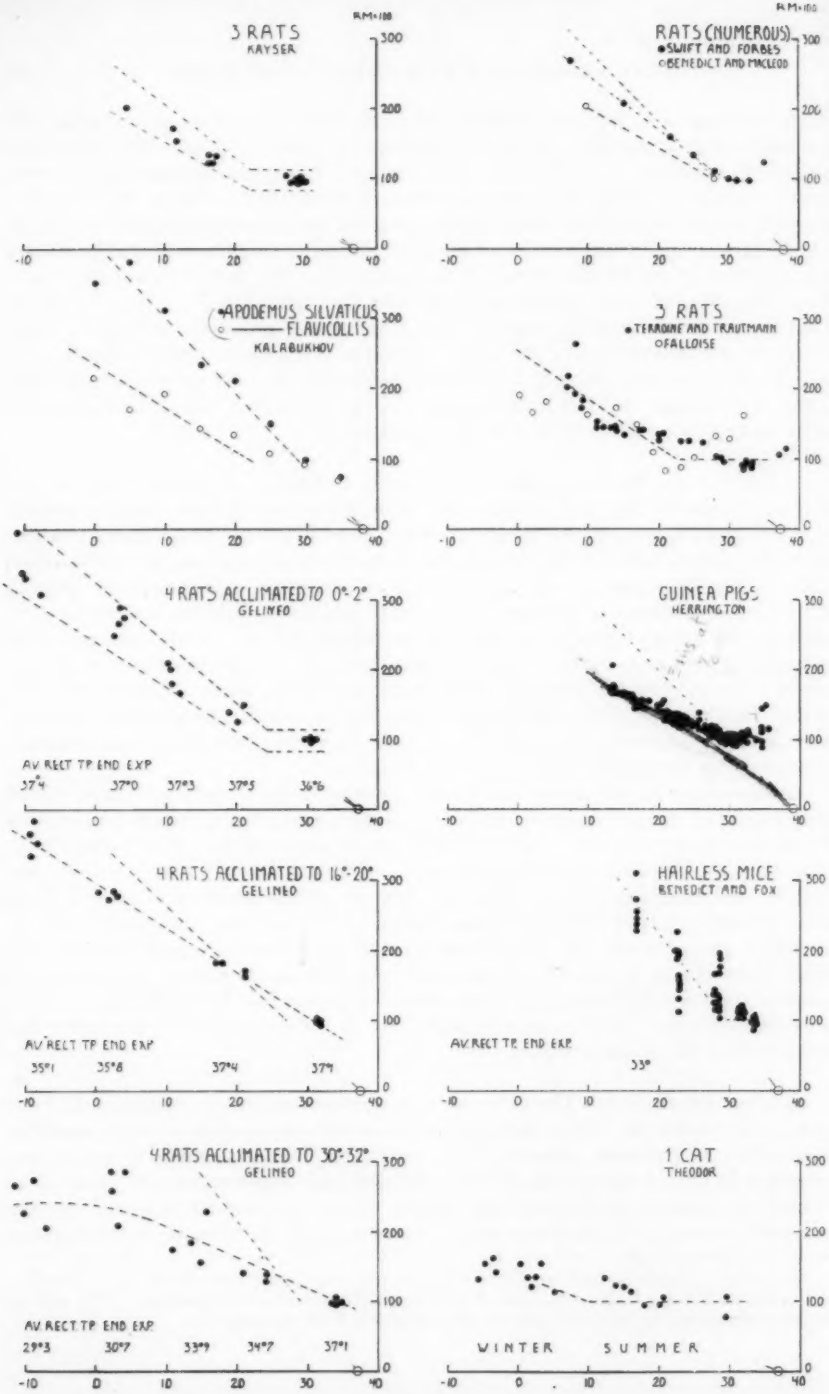
tremes Platypus and Echidna (Martin, 1902) and the Xenarthra, among which are the sloths and armadillos (Wislocki and Enders, 1935). Practically all others regulate to within $\pm 1°$. However, when exposed to decreasing external temperature the central or main body temperature will finally fall, resulting in a relative lowering of the metabolism which drops from the line of proportionality. This is well illustrated in the three series of rat experiments of Gelineo replotted in Figure 8, and occurs in our jungle rat and night hawks (Fig. 6), and in Kendeigh's sparrows (Fig. 9), but is not apparent in Martin's Echidna (Fig. 7). Any change of insulation involves establishment of new temperature gradients and hence some change in the stored heat of the body. In large and well insulated animals, *e.g.*, man in heavy clothing, this may introduce considerable time lag before the new equilibrium is reached. All our animals were relatively small and the arctic ones, especially, were given considerable time for equilibrium.

2. Insulation. Insulation here means the sum total of all factors, such as fur, skin and tissue cooling, and posture, that impede the loss of heat from the animal. In order to obtain proportionality between heat production and temperature gradient below the critical temperature, the insulation must remain constant at its maximum value. This, however, is only approximate. During an experiment the animals make various changes in posture. They have periods of activity which not only influence the energy output, but also the overall insulation. In the numerous experiments where each curve is based on many animals, considerable spread of data can be expected because different individuals may have different critical temperatures. Furthermore, if the experiments have been conducted over a long period of time involving seasonal changes in the animals' insulation (moulting), we must expect only poor correlation (Theodor's cat, Fig. 8).

In all animals the insulation cover over different parts of the body varies within wide limits. Provided, however, that each of these parts at the critical temperature has developed and maintains its individual maximum insulation, it will essentially, from then on, lose heat in proportion to the gradient according to Fourier's law (McAdams, 1942), and hence Newton's law of cooling would still apply for the whole animal. As we shall see, Newton's law of cooling does hold pretty well for the whole animal below the critical temperature, and apparently each external part of the body is able to maintain a maximal insulation and a certain minimum temperature until the environmental temperature drops too low for maintenance of the body temperature. This is also indicated by the observations on the gull's feet mentioned in the previous paper.

3. Heat Production. The extra heat production required to maintain the body temperature below the critical temperature is mainly derived from muscular metabolism, either as shivering, gross activity, or both. It is essentially discontinuous, occurring in bursts, and as the animals may also have other reasons to move about during the experiments we must accordingly expect a considerable spread of data. It should be emphasized that our animals were confined in relatively small cages so

FIGURE 7. Metabolic heat regulation in mammals recalculated from literature. There is a fair overall adherence to Newton's law of cooling below the critical temperature. The echidna shows fair proportionality to gradient in spite of falling body temperature.


3 RATS
KAYSER
RATS (NUMEROUS)
●SWIFT AND FORBES
○BENEDICT AND MACLEOD
●APODEMUS SILVATICUS
○——— FLAVICOLLIS
KALABUKHOV
3 RATS
●TERROINE AND TRAUTMANN
○FALLOISE
4 RATS ACCLIMATED TO 0°-2°
GELINEO
AV RECT TP END EXP
37°4 37°0 37°3 37°5 36°6
GUINEA PIGS
HERRINGTON
4 RATS ACCLIMATED TO 16°-20°
GELINEO
AV RECT TP END EXP
35°1 35°8 37°4 37°1
HAIRLESS MICE
BENEDICT AND FOX
AV RECT TP END EXP
33°
4 RATS ACCLIMATED TO 30°-32°
GELINEO
AV RECT TP END EXP
29°3 30°7 33°9 34°7 37°1
1 CAT
THEODOR
WINTER
SUMMER

that they could not perform any normal exercise in order to keep warm, like running around. This undoubtedly curbs their heat production and gives a false impression of their full natural capacity for metabolic heat regulation.

Experimental Results

All of our own data, and all data from the literature that are sufficiently detailed, have been computed relative to a basal metabolic level in the thermo-neutral zone which we call 100, and are presented in Figures 4 through 9. Wherever possible, lines have been drawn according to the ideas put forth in Figure 3, so as to best fit the experimental data. It is evident that in the great majority of cases, going from arctic through temperate to tropical mammals and birds, the data fit the theory quite well. For a number of animals, especially in the arctic (fox, eskimo dog, polar bear, ground squirrel, arctic gull, snow bunting) and temperate regions (dog, rabbit, guinea pig, and goose), we see that there is a well defined zone of thermoneutrality extending in some of them down to − 30° C. This means that the insulation alone has compensated for the increasing gradient by a steady increase. From the observation that arctic dogs, foxes, and gulls sleep on the snow at − 40° C. to − 50° C.,

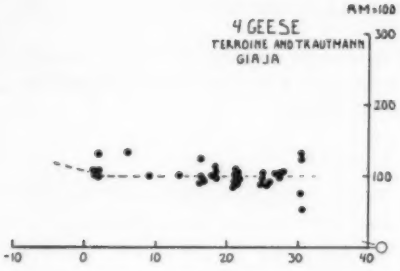
Table I

Simultaneous temperature measurements in adult eskimo dogs (All temperatures Centigrade)

Dog	A	B	C	D	E
Rectal	38.3	38.3	38.2	38.4	40
Subcutaneous	37.2	37.1	37.6	37.4	38.2
Surface of skin	33	36	36.7	35.4	30.0
Outer limit of fur	10	6 to 13	26	23	−10
Air	−3	−3	−3	−3	−19

it is likely that their zone of thermoneutrality extends at least that far, which means that their changing heat dissipation alone can balance the heat loss from + 30° C. to − 40° C. If the foxes' body temperature is 37° C. this is equivalent to an 11-fold change in temperature gradient, and hence they can produce an 11-fold change in heat dissipation in spite of being confined in a small cage. Conversely of course, it follows that at − 40° C. they can increase their heat loss by at least eleven times the basal heat production in order to get rid of heat produced while running. While running freely they can probably dissipate heat considerably better, which may be very important at less severe temperatures. In other words, from their extended zone of thermoneutrality it directly follows that they are equally amazing in ability to dissipiate heat. A major factor in this ability must be vasodilatation and rise of skin temperature. An observation on eskimo dogs brought this out clearly. At − 3° C. air temperature the dogs could maintain a fur surface temperature, for some time, as high as + 26° C. (Table I). An eskimo dog that was brought to the States

Figure 8. Metabolic heat regulation in mammals recalculated from literature. The metabolic rate drops off with falling body temperature in Gelineo's acclimation experiments. In the large rat series by Benedict and MacLeod and the guinea pig series by Herrington there is a poor correlation to the gradient.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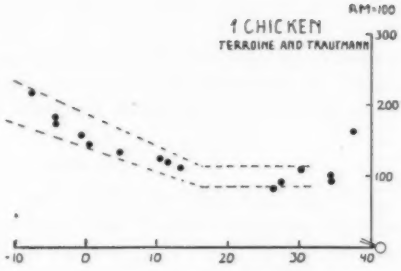
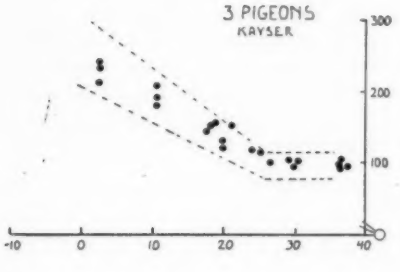
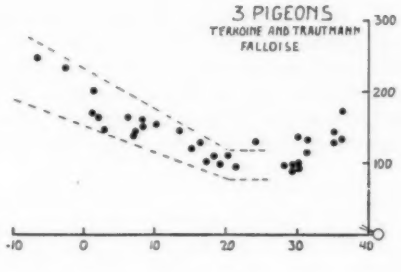
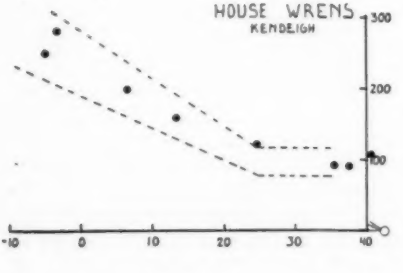
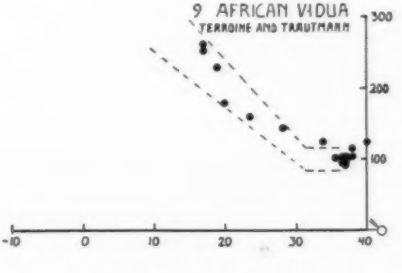
4 GEESE
TERROINE AND TRAUTMANN
GIAJA
300
200
100
-10
0
10
20
30
40

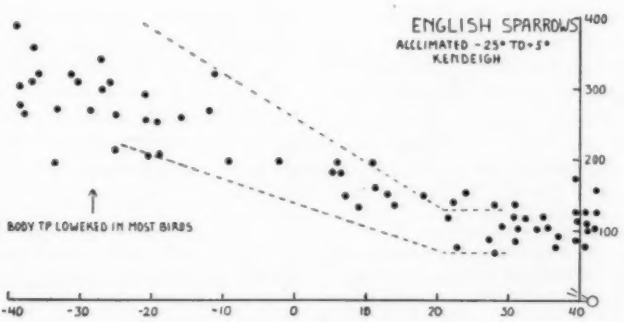

BM=100
1 CHICKEN
TERROINE AND TRAUTMANN
300
200
100
-10
0
10
20
30
40


3 PIGEONS
KAYSER
300
200
100
-10
0
10
20
30
40


3 PIGEONS
TERROINE AND TRAUTMANN
FALLOISE
300
200
100
-10
0
10
20
30
40


HOUSE WRENS
KENDEIGH
300
200
100
-10
0
10
20
30
40


9 AFRICAN VIDUA
TERROINE AND TRAUTMANN
300
200
100
-10
0
10
20
30
40


ENGLISH SPARROWS
ACCLIMATED -25° TO +5°
KENDEIGH
BODY TP LOWERED IN MOST BIRDS
400
300
200
100
-40
-30
-20
-10
0
10
20
30
40

in the heat of the summer got rid of his heat by stretching out on his back, exposing his thinly haired abdomen, and panting vigorously. The observed magnitude of change of heat dissipation in the fox matches the observation by Brody and Cunningham (1936) that the maximum steady state of muscular activity in man and horse is of the order of 10–15 or even 20 times the resting metabolism. It was pointed out in the previous paper that the poorly insulated legs of the arctic mammals and birds are probably a major factor in the heat regulation of these animals.

In most of the tropical animals the zone of thermoneutrality is so limited and transitory that the two parallel lines drawn in the diagrams represent more of an abstraction than a reality. In some cases the transition between the zone of thermoneutrality and metabolic rise is definitely a curve (see Goto's rats, Herrington's mice, Fig. 7). Such a thing would happen also in our insulated model if the metabolic rise started before the maximum of the insulation had been reached. This may be a reason for the low slope in the rat experiments of Benedict and MacLeod and of Swift and Forbes (Fig. 8), and in the guinea pig experiments of Herrington (Fig. 8). Another reason may be a possible drop in body temperature at the lowest temperatures. Such a drop of the curves due to fall in body temperature is illustrated in Gelineo's rat experiments (Fig. 8), in Kendeigh's sparrows (Fig. 9), and in our jungle rat and night hawks (Fig. 6).[8] In the scant data on the platypus and the three Australian marsupials (Martin, Fig. 7), there is an upward trend in the curves. Because of the inherent spread in all similar experiments it seems doubtful that this trend is significant, but it could happen if the insulation deteriorated markedly with activity as may occur with human clothing.

As has been described, there is a considerable spread in most of the data and also a tendency to proportionately greater spread during the period of metabolic heat regulation, when bursts of activity rather than an even flow supply the heat which maintains the body temperature. The maximum amount of heat produced above the base value is undoubtedly influenced by time, and in long-time experiments the peak metabolism is seldom over 400 (Echidna) and usually not more than 300 per cent. In most cases, however, the high peak values (*metabolisme du sommet*) found after acute chilling in ice water (Giaja, 1925) cannot be produced in prolonged experiments. Animals at liberty to move about freely can, however, undoubtedly maintain a higher metabolic rate than under the cramped conditions of an experiment.

With the facilities available at the Arctic Research Laboratory at Point Barrow we were not able to reach the critical temperature for the foxes or dogs. However, with excellent cooperation from the Navy, two foxes and several snow buntings were flown to the Naval Research Laboratory, Washington, D. C., and a series of observations was made at temperatures down to − 80° C. (Table II). From the table it is apparent that − 70° C. induced shivering and is therefore below the critical temperature of the fox. He suffered no loss of body temperature after one hour's

[8] The drop in body temperature and poor metabolic response to the lowered outside temperature observed in this goatsucker may well be due to a hibernation reaction, as the related poor-will (*Phalaenoptilus nuttallii*) is known to hibernate (Amadon, 1949). This information was kindly furnished by Dr. R. C. Murphy of the American Museum of Natural History.

FIGURE 9. Metabolic heat regulation in birds recalculated from literature. Many of Kendeigh's sparrows drop off from the line of proportionality because of drop in body temperature, and many died after the severest cold exposure. *Vidua paradisea* (Terroine and Trautmann, 1927) is a bird native to equatorial West Africa (Delacour and Edmond-Blanc, 1933, 1934).

TABLE II

Rectal temperature in white fox subjected to low temperature
(All temperatures Centigrade)

Air temp.	Duration in minutes	Rect. temp. before exp.	Rect. temp. end exp.	Remarks
+22		37°7		
−20		37°7		
−50	120	37–39°	37°6	Lying down asleep.
−60	105	37–39°	39°	Lying down asleep.
−70	90	39°	37°3	Licked feet ½ hour, curled up asleep, shivering after 1 hour.
−80	60	37–39°	38°	Licked himself, asleep, shivering after 5 minutes. Legs stiff, active.

stay at − 80° C., and his metabolic heat regulation can probably balance considerably lower temperatures, but he may suffer local frost injury long before exhaustion of his metabolic resources. From observations of sleeping foxes and eskimo dogs it is believed that their critical temperature is somewhere between − 45° C. and − 50° C., and it is most likely the same in all the other larger mammals of the same climate. Snow buntings, which normally migrate south in the winter, could not take − 50° C. and their body temperature dropped seriously within an hour.

The data on all our arctic and tropical animals are collected in charts, Figures 10 and 11, where the full drawn lines represent observations and the dotted lines represent extrapolation of the metabolism below a known critical temperature.

The animals most sensitive to low temperature have the steepest curves, with critical temperatures near the body temperature. The animals hardiest toward cold have the least slope, with critical temperatures farthest from the body temperature. We have noted that the cold hardiest animals, the fox and eskimo dog, can be expected to tolerate the coldest temperatures on earth merely by increasing their me-

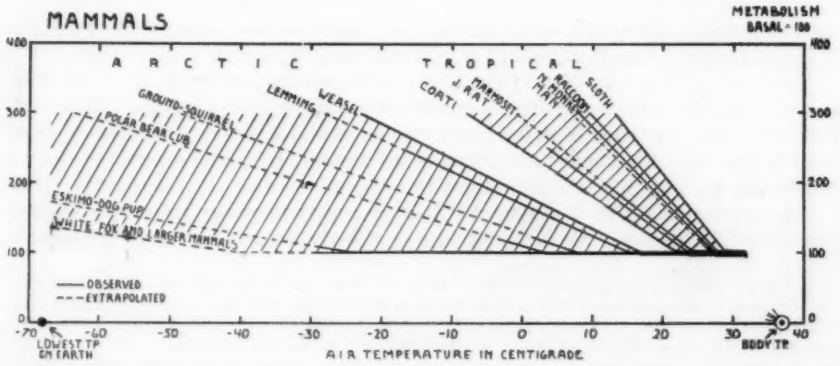


FIGURE 10. Heat regulation and temperature sensitivity in arctic and tropical mammals based on plots in Figures 4, 5, and 6. The fox needs only slight increase in metabolic rate to stand the coldest temperature on earth. The critical gradients (tp sensitivity) and the slope of the curves depend on the product of the basal metabolic rate and the overall body insulation.

tabolism 30–40 per cent, whereas the same increase in metabolism in a tropical animal will only help him by a few degrees.

The critical temperature in naked man is known to be around 27° C. (DuBois, 1936) to 29° C. (Winslow and Herrington, 1949), which places him among the more temperature sensitive of the tropical mammals. We have no data as to the critical temperature of Eskimos. They wear just as warm clothing as white men in the same environment, often warmer, and it is likely that the Eskimo is as sensitive to temperature as other people that wear clothing. Man is indeed a tropical animal carrying his tropical environment with him.

By using different amounts of clothing, tropical man can span the whole range from the tropics to the arctic and it could be assumed that his energy requirements below the critical temperature for each clothing assembly would essentially follow

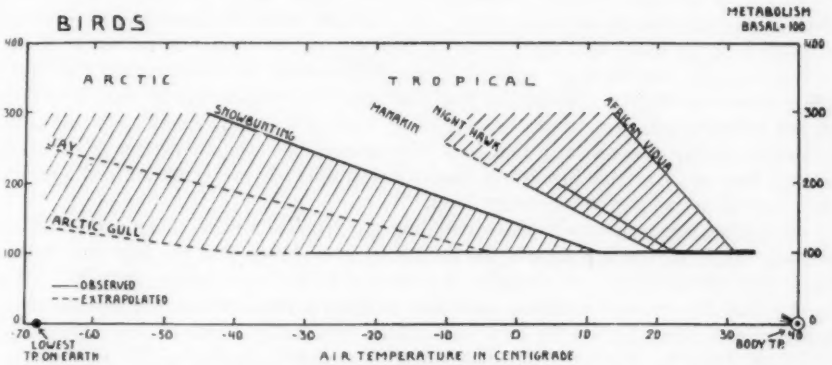


FIGURE 11. Heat regulation and temperature sensitivity in arctic and tropical birds, based on plots in Figures 5, 6, and 9.

Newton's law of cooling. It seems a challenge that we have been unable to unearth in the literature experiments in man, naked or clothed, with enough data to demonstrate adequately the thermoneutral zone and the slope of energy expenditure beyond it. In Figure 10 man's line below the critical temperature is hence dotted. It is known however, from studies at the Climatic Research Laboratory, Lawrence, Massachusetts, that the insulation value of clothing may drop considerably (50 per cent) during exercise (Belding, 1949, and personal communication), which would move the curves upward. Man cannot usually spend a night at − 40° C. in one-layer arctic fur clothing or naked in a one-layer arctic fur sleeping bag without getting cold and shivering. He needs more insulation than the animals whose fur he is using. The reasons may be several. In his outstretched sleeping position he exposes much more surface than an arctic mammal sleeping curled up. The arctic mammals may tolerate a low leg temperature, like the arctic gull, and thereby greatly conserve heat. Fur clothing and fur sleeping bags are usually not made from the heaviest winter fur, but rather from lighter autumn fur with less insulating value.

It is readily seen by comparing Figures 10 and 11 of this paper with Figure 3 in the preceding paper that the correlation between the measured insulation of a piece of fur of the animal and its temperature sensitivity does not hold in detail.

Thus the smaller arctic animals are considerably less temperature sensitive than the equally well or better insulated tropical mammals.

From Figure 3 it is clear that the temperature sensitivity, *i.e.*, critical gradient, depends upon the product of the overall insulation and the basal metabolic level. The overall insulation is only roughly reflected by measurements of the fur insulation on a patch taken from the body side or back, but even with this limitation we shall see that most of the apparent discrepancies can be explained by considering the metabolic level.

Summary

A series of arctic and tropical mammals and birds at Point Barrow, Alaska (lat. 71° N.) and in Panama (lat. 9° N.) was subjected to various air temperatures in a respiration chamber where the heat production was determined by oxygen consumption or carbon dioxide production. The larger arctic mammals and birds showed no increase in metabolism at − 30° C. and from observations on sleeping animals it is probable that their zone of thermoneutrality extends to − 40° C. or − 50° C. The smaller arctic species show a high critical temperature and the tropical species even higher. Metabolic heat production increases rapidly with lowering of the temperature in a tropical mammal or bird, and slowly in an arctic animal. It can be shown theoretically that in a thermoregulated system with a fixed basal energy level and variable insulation the critical gradient is proportional to the maximal insulation and the basal energy level.

In a large series of experiments including our tropical and arctic animals, and all animals affording enough data in the literature, it is shown that the heat loss below the critical temperature is essentially proportional to the body-to-air gradient. This means that the overall insulation evidently reaches a maximum at the critical temperature and from then on the heat loss follows essentially Newton's law of cooling. It follows from this that an arctic mammal with a critical gradient of 70° C., by doubling its metabolism, theoretically would double the gradient. Only 40 per cent increase of its metabolism (or insulation) would suffice to take it down to − 70° C. which is near the lowest recorded temperature on earth.

The very broad zone of thermoneutrality in the larger arctic species, from + 30° C. to − 40° C., shows their ability to balance an 11-fold increase in gradient and hence the animal can change its heat dissipation by a factor of 11 even when lying down. It is believed that vasomotor control of the poorly insulated legs must play an important role in the general thermoregulation of these animals.

In the tropical mammals and birds the critical gradient is low, often only 10° C., which makes them sensitive to even small temperature changes. A 10° lowering of the air temperature from the critical temperature doubles the gradient for the tropical mammal; a 9° increase decreases the gradient 10 times, and in order to maintain the body temperature tropical animals must be able to adjust insulation and metabolism in the same proportion as the gradient. They are thus extremely sensitive to temperature changes.

The whole range of heat regulation from tropical to arctic mammals and birds is represented on two charts, Figures 10 and 11.

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us by Captain H. W. Taylor, USN, Commanding Officer of the U. S. Naval Air Station, Coco Solo, Canal Zone. We are also greatly indebted to Mr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone, for helping us in every way at the Barro Colorado Laboratory. We are grateful to Mr. Walter Flagg and to Mr. R. H. Stapleton for much capable assistance and to Dr. Harald Erikson for permission to use some of his ground squirrel data. While preparing the data we have had the privilege of stimulating discussions with Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton.

Addendum

After the manuscript and illustrations were all prepared, Mr. Charles M. Bogert at the American Museum of Natural History kindly made us aware of an important paper appearing in the latest available issue of *Bullétin de l'Académie des Sciences de l'U.R.S.S.*, Volume 2, 1947, by R. P. Olnianskaya and A. D. Slonim. They subjected several zoo animals at Leningrad to temperatures from + 20° C. to − 20° C., measuring metabolism and the rectal temperatures: *Vulpes vulpes, V. lagopus, V. melanotus, Lepus europaeus, L. timidus,* and *Nyctoreutes procyonoides.* The polar fox showed only insignificant rise in metabolism from + 20° C. to − 20° C., the red fox a 50 per cent rise, and the other animals more. There is a fair correlation between gradient and metabolic rate in their curves. Absolute metabolic rates are not given. The authors conclude that the polar species have a more constant body temperature at low environmental temperatures than the southern species, and that the metabolism rises only little in the polar species as compared with the southern species. The maintenance of constant body temperature in the arctic species is considered to be due to an extraordinary vascular control. Insulation is not mentioned.

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ADAPTATION TO COLD IN ARCTIC AND TROPICAL MAMMALS AND BIRDS IN RELATION TO BODY TEMPERATURE, INSULATION, AND BASAL METABOLIC RATE *

P. F. SCHOLANDER,[1] RAYMOND HOCK,[2] VLADIMIR WALTERS,[3] AND LAURENCE IRVING [4]

In two previous papers [5] we showed how climatic adaptation of arctic and tropical mammals and birds was clearly manifested by their widely different critical gradients. The critical gradient was defined as the largest temperature difference between the body interior and the air that an animal can stand without losing body temperature, when the metabolism is basal.

It was pointed out that the critical gradient in an insulated thermoregulated system depends upon the product of the over-all insulation and the basal metabolic rate. This leaves the animals with three possible main avenues for adaptation to cold, namely (1) the body-to-air gradient, (2) the insulation, and (3) the basal metabolic rate, and it is our task in the present paper to estimate quantitatively the importance of each of these factors for climatic adaptation. For this purpose we must first know more precisely what thermal stresses animals are faced with in their arctic and tropical climate.

The Thermal Environment

The temperature conditions at Point Barrow and at Barro Colorado are about as different as could be found. Records shown in Table I indicate that on the arctic coast annual extremes may differ by 65°. Inland or in exceptional years on the coast the difference may be 80°–90°.

At Barro Colorado the temperature in a shaded location near the ground is extremely constant and seldom varies from 28° C. Tropical microclimates, on the other hand, vary considerably, as is shown by observed diurnal variations with altitude in the forest at Barro Colorado. In February the diurnal variation at ground level was 6.5°, at a 55 foot height 9.5°, and at an 86 foot altitude 16.5° (Allee, 1926). The mean maximum-minimum range at ground level is 2.9° and in the canopy 11.8° or four times greater.

On first consideration we would expect an arctic mammal to be cold adapted and a tropical mammal to be heat adapted. It is quite obvious that the arctic mammals are adapted to withstand cold or they would freeze to death. If we consider, how-

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[5] Many problems touched upon in this and the foregoing papers have been discussed in the stimulating paper "The application of the theory of heat flow to the study of energy metabolism" by Burton (1934).

ever, that fur always conserves heat and that most tropical mammals are fur covered, then we realize that conservation of heat, *i.e.*, adaptation to cold, is also a conspicuous feature in tropical mammals. We may say then that an arctic mammal is adapted to cold and a tropical mammal to less cold. That this is true is evident if we consider the critical gradients. Several of the tropical mammals examined have a critical temperature between 25° C. and 27° C. which is only 10° below their body temperature. If the air temperature drops only a few degrees lower they begin to shiver from cold, and if it were to rise only 9° above their critical temperature they would have to increase their heat dissipation ten times to keep in heat balance. Such changes in environmental temperature do, however, take place between day and night in the tropics (Allee, 1926). In contrast to this an arctic mammal at its critical temperature of − 40° C. to − 45° C. can adjust to 0° C. merely by halving the insulation. Such large changes in the environmental temperature are seasonal rather than diurnal, however, and are compensated for by seasonal changes in insulation (shedding).

It seems then that the problem for tropical mammals is neither overheating nor cooling but, actually, both. Their low critical gradient, whether due to low insulation or low metabolism, gives them a very small range of temperature tolerance in

TABLE I

Temperature reported at Barrow, Alaska (lat. 71°) in 1948, converted to centigrade degrees (from climatological data, Alaska, 1948)

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Average	−28	−24	−27	−17	−10	− 1	5	2	− 4	−12	−21	−29
Minimum	−38	−42	−43	−32	−26	−10	− 2	− 5	−15	−26	−40	−43
Maximum	− 5	− 4	−13	1	0	4	22	14	5	− 2	− 2	− 8

both directions. The heat of the day, rain and storm, and the cool of the night will frequently exceed their narrow zone of thermoneutrality and in short succession they may be subjected to both cold and heat. The same is often experienced by man in the tropics. The days may be extremely hot even with a minimum amount of clothing, and a night without extra clothes can be very chilling. What then should a tropical mammal adapt for, overheating in day or cooling at night? The fact that most of them are well furred shows that they do adapt to cold. By what means and to what extent they can protect themselves against heat, we have not studied quantitatively. Some of them do it by sweating and panting, many avoid the sun by seeking shade, and many are inactive during the heat of the day; some resort to the water for cooling. It would seem that gains in latitude of heat regulation would be an advantageous adaptation to a tropical climate.

We pointed out above how cold sensitivity would depend upon three main factors: (1) the body-to-air gradient, (2) the insulation, and (3) the basal metabolic rate.

Body Temperature and Climatic Adaptation

We shall first consider the body-to-air gradient in relation to climatic adaptation. As the heat loss is proportional to the gradient, other factors being equal, arctic mam-

mals would gain in heat conservation by having a lower body temperature than other mammals, and tropical mammals would gain in latitude of heat regulation if they were warmer than usual.

We saw in the preceding papers that all arctic mammals and birds so far tested have an internal body temperature like the temperate forms, and actually they would gain very little in heat preservation from a low body temperature unless it were considerably lower than normal. A body temperature of 30° C. instead of 37° C. would, at − 40° C. air temperature, save only 10 per cent of the rate of heat loss. The large lowering of the body temperature during hibernation combined with an insulated nest does, however, reduce the gradient and the heat loss considerably, but it occurs only in dormant animals. The smaller arctic mammals and man could not maintain inactive resting periods without lowering the gradient, however, and they can only do this if they raise the external temperature by providing themselves with added insulation in the form of nests and clothing. It is interesting to note that "behavioral thermoregulation" has also been found to be characteristic for all reptiles thus far studied (Bogert, 1949).

There are no tropical mammals or birds known to have body temperatures higher than usual (Wetmore, 1921; Wislocki, 1933), and consequently none of them have a heat adapted body temperature. Quite a few normally have a low body temperature, however, *e.g.*, sloths, armadillos, anteaters (Wislocki and Enders, 1935), and monotremes (Martin, 1902). Lowered body temperature *per se* makes them a little more cold hardy, but at the same time more sensitive to changes in the air temperature because of the lowered critical gradient. They gain some latitude, however, by the fact that they are all poor temperature regulators. This is a condition well known in bats and other hibernators from all climates and represents traces of a thermal survival latitude characteristic of poikilotherms. It has nothing to do with an adaptive change of body temperature to a new and constant level such as we would expect in a truly homoiothermic animal. We may state then that there are no signs so far that body temperature of mammals and birds is adaptive to the different climates on earth. A logical corollary of this is that it cannot have been adaptive to the over-all climatic conditions on earth either. It seems then that the narrow band of body temperature on which both birds and mammals operate is a fundamental, nonadaptive constant in their biochemical setup. It can be kept constant only within certain climatic limits which are determined by the latitude of the physical and chemical heat regulation of the animal. Whereas mammals and birds undoubtedly could adapt to colder climates than we have today by increasing their insulation, they are very near their upper limit in a warm tropical climate. Many parts of the tropics are so hot and humid that a few degrees' rise in the temperature would mean death for mammals and birds because they cannot adapt to it by raising their body temperature.

In contrast to the inadaptability of the internal body temperature, peripheral parts may show a great deal of temperature adaptation, such as the cold extremities of aquatic mammals and birds.

Insulation and Climatic Adaptation

Having eliminated the internal body temperature as an adaptive factor in arctic and tropical mammals, there remains the insulation and the basal metabolism to ac-

count for the striking differences which we have observed in their sensitivity to cold. It is, of course, common knowledge that the mammalian body insulation is highly adaptive to climate, phylogenetically as well as ontogenetically. We saw that the mammalian species of cold climates have, in general, much warmer fur than those of warm climates; that there are adaptive seasonal changes (moulting), and it is known that, for instance, dogs (v. Hoesslin, 1888) and rabbits (Mayer and Nichita, 1929) can be experimentally induced to increase their insulation if exposed long enough to cold. There is no reason to believe that the arctic climate is so cold that the larger mammals cannot produce adequate insulation against it but must resort to a high metabolism. They could certainly grow and wear much longer and warmer fur if they needed to, *i.e.*, a 500 kg. moose would not need to stop at the insulation of a 5 kg. fox.

In a previous paper we substantiated, by insulation measurements, the general rule that arctic mammals have warmer furs than do the tropical mammals. We found, however, some striking exceptions to this. For instance, the little arctic weasel, although much cold hardier, possesses only half the fur insulation of the tropical sloth. We pointed out that the explanation was to be found in the difference in the heat production of the two animals. The cold legs of arctic aquatic birds and mammals (and probably of the terrestrial forms as well) may be taken as another example of adaptive insulation.

Basal Metabolic Rate and Climatic Adaptation

As pointed out by Rubner (1883), Richet (1891), Voit (1901) and others, practically all mammals so far investigated show a nearly linear relation between the basal heat production and the body surface, usually estimated according to Meeh's formula as $K \times \text{Weight}^{2/3}$. They were found, in other words, to have approximately the same rate of heat dissipation per surface unit, or to follow the "surface law" of Rubner. The material was later amplified by Benedict (1938) in his famous mouse to elephant curve, and it was realized that the correlation came closer to $K \times \text{Weight}^{3/4}$ (Kleiber, 1932, 1947; Brody and Procter, 1932), which does not have the meaning of a surface area, although it is numerically almost the same except at the extremes of size. The fact that a near surface relation between body size and basal metabolic rate holds also for many groups of cold-blooded animals (Weymouth, Crismon, Hall, Belding and Field, 1944) is a clear indication that this relation is fundamental and is not primarily caused by heat loss (Terroine and Delpech, 1931; Krogh, 1941; Zeuthen, 1947).

From Benedict's mouse to elephant curve it is indicated that the basal metabolic rate is determined by body size, irrespective of climate. If this is so, however, and the body temperature is constant, then we may conclude that the only factor left to take care of climatic adaptation is insulation. Without further evidence we cannot preclude, however, that one might still find different adaptive metabolic levels in animals living under such extremes as the arctic and the tropics. A higher heat production, other factors being equal, would make an animal cold-hardier, and a lower heat production heat-hardier, and it is of interest therefore to know the metabolic rate of our animals in absolute units as compared with animals from less extreme climates.

In Figures 1 and 2 we have plotted our arctic and tropical mammals on the stand-

ard mouse to elephant curve, drawn according to the equation Cal./per day = 70 kg.$^{3/4}$. The metabolism has been calculated from the oxygen consumption (arctic) or the CO_2 production (tropical), assuming an RQ of 0.80, and a caloric equivalent of one liter of oxygen = 4.8 Calories. Generally when such determinations are made the animal is confined in a cage but can move about during the experiment if it so wishes. Even if active runs are weeded out, the strict prerequisites for a basal determination as in man rarely apply. However, we believe that our determinations fall in line with most other determinations of the basal energy level in wild animals, with the added advantage that we know definitely that each determination was made in the thermoneutral zone. Most of our determinations were started in the morning, the larger animals having fasted over night, the smaller ones for a couple of hours.

From Figure 2 we see that our adult arctic mammals, lemmings, ground squirrels, and foxes all line up closely with the standard curve, but the weasels do not. Since the larger arctic mammals (from a 5 kg. fox to a 500 kg. moose) have about the same

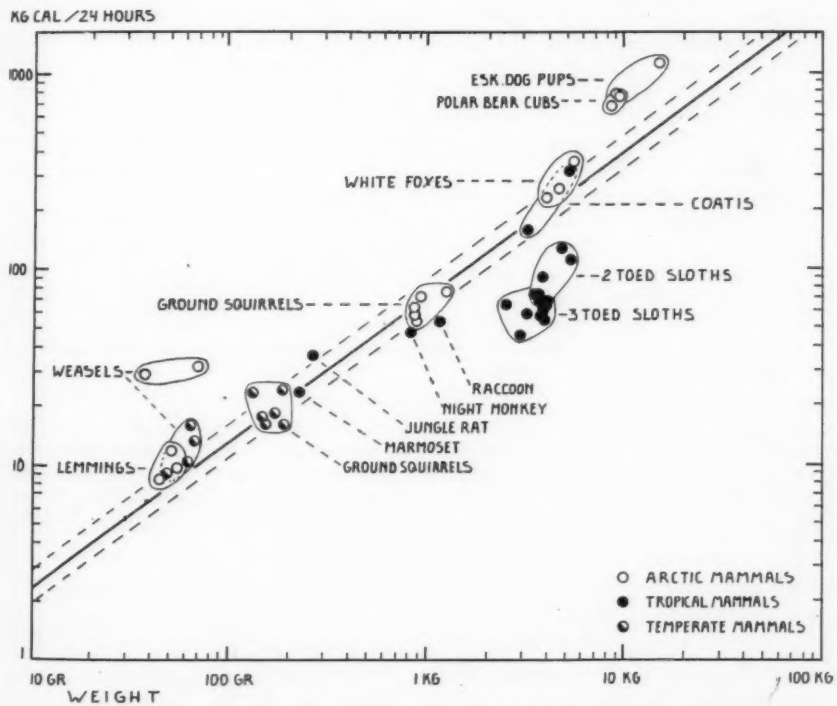

FIGURE 1. Basal metabolic rate in relation to body size. Full drawn line is the mouse to elephant curve drawn according to the equation Kg. Cal./day = 70 × kg.$^{3/4}$. Dotted parallel lines are 20 per cent deviations. Besides our own material the following has been used: ground squirrels (Erikson, 1950; and Gelineo, 1939), sloths (Ozorio de Almeida and Branca de Fialho, 1924; Irving, Scholander, and Grinnell, 1942), least weasels from Wisconsin (Morrison, unpublished).

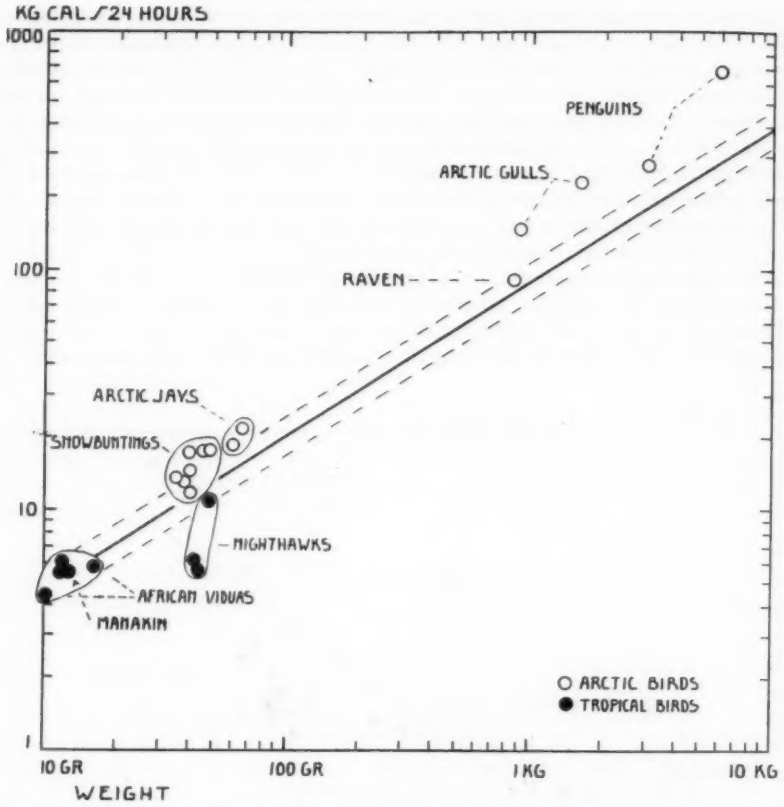

FIGURE 2. Basal metabolic rate in relation to body size. Full drawn line represents the curve given by Brody (1945) for birds according to the equation Kg.Cal./day = $89 \times \text{kg.}^{0.64}$. Dotted parallel lines are 20 per cent deviations. The African *Vidua paradisea* are taken from Terroine and Trautmann (1927), the antarctic penguins (*Pygoscelis papua* and *Eudyptes chrysolophus*) from Scholander (1940). The raven was kept most of the time as a pet in the laboratory building (− 5° C. to + 25° C.).

fur insulation per unit surface, and since they are subjected to the same climatic temperature gradients, it follows that their heat loss will be roughly proportional to their surface area. Since the fox falls on the mouse to elephant line it is therefore likely that all the larger arctic mammals do also. We see further that the tropical mammals, excepting the two species of sloths, likewise fall on the line. If we realize furthermore that the mouse to elephant curve already is based on animals ranging from relatively cold-hardy temperate rabbits to tropical elephants we may state as a tentative generalization that *the basal metabolic rate of terrestrial mammals from tropics to arctic is fundamentally determined by a size relation according to the*

formula Cal./day = 70 kg.$^{3/4}$, and is phylogenetically nonadaptive to external temperature conditions. Equally nonadaptive is the body temperature, and the phylogenetic adaptation to cold therefore rests entirely upon the plasticity of the factors which determine the heat loss, mainly the fur insulation. Small adaptive changes cannot, of course, be detected by such interspecific comparisons as these. There is also, however, intraspecific evidence from observations on man, which have failed to reveal any certain racial metabolic or body temperature adaptations to cold climates (DuBois, 1936). Man and animal alike do it all with insulation.

Our weasels were adults and had a metabolic rate two to three times higher than that expected from the standard curve. There are indications that our weasel figures are representative. The figures are averages of the lowest of several determinations in which the weasel was seen to be lying down curled up. Furthermore, during cold experiments they mobilized a heat production nearly four times larger than basal, which is as high as any other animal could maintain for long periods in a small cage. Finally, as we shall see, the high metabolism is a corollary to their relatively poor body insulation. Dr. Peter R. Morrison has kindly informed us that the average minimum value from runs on two individuals (*Mustela rixosa*) from Wisconsin showed only a slight elevation above the standard curve (Fig. 1). Hence we are forced to conclude that our arctic weasels had a greatly increased resting metabolic rate compared with the southern form. This may possibly mean a metabolic adjustment to cold. If the cold were the direct reason for the high resting rate it seems somewhat odd that the fur is so slight; presumably it could easily have been doubled!

We must still account for the fact that some tropical mammals have a very low metabolic rate, notably the sloth and possibly the armadillo. The sloth is clearly adapted to cold because of its warm fur, which acts compensatory to its low metabolism. Its low body temperature is likewise heat conserving. Low metabolism combined with low or labile body temperature is found also in non-tropical mammals, *e.g.*, marmot (Benedict, 1938), bats (Hock, 1949) and others and has scarcely anything to do with climatic adaptation but is rather tied up with the hibernation reaction which occurs in all climates.

In birds we do not know the relation between basal metabolic rate and weight as well as in mammals. In Figure 2 our few arctic and tropical birds have been plotted upon the standard exponential curve given by Brody (1945), based on domestic birds (hens, pigeons, sparrows, canaries). The snow buntings migrate south in the winter and their high critical temperature (+ 10° C.) indicates that they were under continuous cold stress during all of the winter at Point Barrow. Nevertheless their basal metabolism was not elevated. Our two gulls were high, and normally they also migrate to warmer climates in the winter. In a series of large birds, Benedict and Fox (1927) found the highest figures for pelicans and gulls. Scholander (1940) found high figures for penguins, and many aquatic mammals are also high (Fig. 3). In Benedict and Fox a series of large birds from the New York Zoological Park includes many tropical species and they fall near the standard line, together with the temperate birds. Two of our night hawks are very low, and this is probably due to their faculty of hibernation. More material is necessary before we can tell whether the basal metabolism in birds may be adaptive to climate.

It should be pointed out that ontogenetic adaptations of the basal metabolic rate

to cold have been induced experimentally in several species of mammals and birds (Gelineo, 1934, 1939; Schwabe, Emery and Griffith, 1938; Ring, 1939; Lee, 1942). The rise is generally below 20 per cent, but can get up to 50 per cent, and depends upon the length of exposure and the degree of cold. It is not clear whether the rise would be permanent. It would seem likely that in some animals at least, within reasonable limits and given enough time, the insulation would take over the whole adaptation, permitting the metabolism to drop back to normal. In any case a mere 50 per cent increase in the metabolic rate is an ineffective and expensive adjustment to a gradient which may have changed 10 times or more! We did not find a high basal metabolic rate in pigeons which had been kept for four months at Point Barrow at 0° C. to − 40° C. air temperature.[6] Dugal and Thérien (1947) found that rats and guinea pigs needed large quantities of ascorbic acid to adapt to cold, *i.e.*, to thrive and grow at a body-to-air gradient which was two to three times larger than

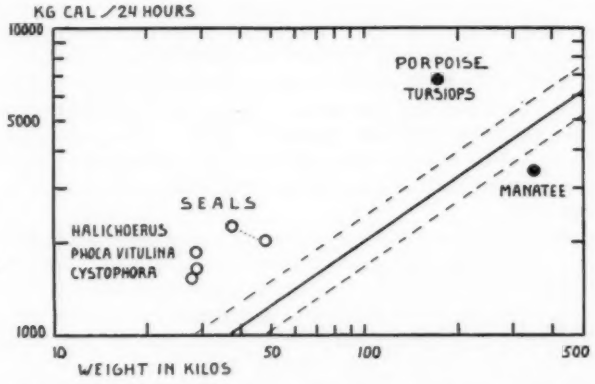

FIGURE 3. Basal metabolic rate in relation to body size in some aquatic mammals. Full drawn line is mouse to elephant curve, with 20 per cent deviation lines dotted (cf. Fig. 1). The seal records are taken from Irving, Solandt, Solandt, and Fisher (1935) and Scholander (1940), the porpoise from Irving, Scholander, and Grinnell (1941), and the manatee from Scholander and Irving (1941).

that of the control animals. The cold-adapted animals consumed only about 40 per cent more food than the controls, however, and it therefore seems that the major factor in their cold adaptation must have been a decrease in their heat dissipation. The diminished heat loss could have been due to growth of more fur, establishment of colder and deeper tissue gradients, difference in posture, etc.

Very feeble seasonal changes of basal metabolism (± 5 per cent) have been observed in pigeons (Dontcheff and Kayser, 1934) which may, however, have been caused by factors other than temperature. In the regular and fully developed seasonal adaptation to cold which we see in our wild species, it seems likely that the

[6] Pigeons are known to stand considerable cold (Horvath, Folk, Craig and Fleischmann, 1948) and a number of them were kindly sent to us from Edgewood, Maryland, at the suggestion of Dr. David Bruce Dill.

insulation is normally responsible for the whole temperature adaptation. Ontogenetic changes in the basal metabolic rate of man, induced by seasons or transfer to hot or cold environment, are small and difficult to interpret (DuBois, 1936; Ames and Goldthwait, 1948); man does it mainly by insulation.

The Relation of Cold Hardiness to Insulation and Basal Metabolism

The adherence of mammals from all climates to the mouse to elephant curve holds astonishingly well in general, but there are marked exceptions. It applies only to adult animals, young ones being generally high (cf. Brody, 1945, p. 406). This undoubtedly is the explanation for the high basal rates of our very young polar bear cubs and husky pups. There are also well known examples of animals with substandard metabolic rate, notably the marmot (Benedict, 1938) and the sloth (Ozorio de Almeida and Branca de Fialho, 1924).

For all these animals, whether their metabolic rate falls on the standard line or not, heat output must balance the heat production, provided they maintain the body tem-

TABLE II

Basal metabolism of some arctic and tropical mammals and birds

The basal rate (BM) is the lowest range of the resting rates (RM) given in Figures 4, 5, and 6 of preceding paper

$BM = f \times RM$

Species	Weight grams	f	BM kg. cal./ 24 hrs.	Species	Weight grams	f	BM kg. cal./ 24 hrs.
Arctic mammals				Arctic birds			
Dog (pups)	14,500	0.85	1,140	Gull	1,600	1.0	304
Dog (pups)	9,000	0.85	930	Raven	850	1.0	92
Polar bear (cubs)	9,300	1.00	750	Pigeon, domestic	510	1.0	33
Polar bear (cubs)	8,500	1.00	690	Pigeon, domestic	400	1.0	28
White fox	5,500	0.85	353	Pigeon, domestic	375	1.0	47
White fox	4,600	0.85	268	Pigeon, domestic	300	1.0	49
White fox	4,000	0.85	234	Pigeon, domestic	400	1.0	49
Ground squirrel	1,250	0.70	77	Alaska jay	65	1.0	22
Ground squirrel	940	0.70	72	Alaska jay	62	1.0	18
Ground squirrel	880	0.70	55	Snow bunting	35	0.75	14
Ground squirrel	880	0.70	67	Snow bunting	48	0.75	18
Ground squirrel	870	0.70	59	Snow bunting	38	0.75	13
Weasel	70	0.85	31	Snow bunting	48	0.75	18
Weasel	38	0.85	29	Snow bunting	40	0.75	17
Lemming	56	0.9	9.6	Snow bunting	40	0.75	14
Lemming	52	0.9	12	Snow bunting	40	0.75	12
Lemming	45	0.9	8.8				
Tropical mammals				Tropical birds			
Coati	5,100	1.0	335	Night hawk	43	1.0	7.7
Coati	3,200	1.0	163	Night hawk	43	1.0	7.6
Two-toed sloth	3,770	1.0	71	Night hawk	49	1.0	13
Raccoon	1,160	1.0	53	Manakin	12	1.0	6.4
Night monkey	820	1.0	48				
Jungle rat	265	1.0	39				
Marmoset	225	0.9	27				

perature constant, and we should expect that animals with the same critical gradient, living under the same climatic conditions, would show an inverse relation between the metabolic rate and the insulation according to the formula $\Delta\ TC$ = Insulation × basal metabolism. If we compare our weasels with the "standard" lemmings (Fig. 1) we see that the weasels have two to three times as high basal rate, but only half the insulation, and we may therefore say that the weasel requires less insulation *because* its metabolism is so high. Our little polar bear cubs and husky pups were observed to have much shorter fur than the adults, and evidently needed less insulation *because* of their high metabolic rate.

The sloth, with less than half the standard metabolism, has a fur nearly twice as warm as other tropical mammals (for instance, the coati) but even so its metabolism is barely enough to keep it warm, as evident from its extreme temperature sensitivity.

TABLE III

Critical gradients (ΔTC) observed from respiration curves compared with gradients calculated from measurements of fur insulation and basal metabolism (all temperatures centigrade)

Arctic species	White fox	Weasel	Lemming	Ground squirrel (Summer)
Observed ΔTC	80°	22°	20°	20°
Calculated ΔTC	68, 82, 70	40, 27	13, 13, 18	17, 18, 19

Tropical species	Coati	Jungle rat	Marmoset	Night monkey	Raccoon	Two-toed sloth	Three-toed sloth
Obs. ΔTC	15	14	13	10	9	8	—
Cal. ΔTC	13	8	11	11	5	9, 10, 7, 8	6, 6, 9, 5

It seems that another tropical mammal, the armadillo, evidently can have a basal metabolic rate as low as that of the sloth, although it is almost naked. Ozorio de Almeida and Branca de Fialho (1924) found practically normal basal rates in six specimens, and Scholander, Irving and Grinnell (1943) found only half of that rate in three specimens of the same species. Whatever the reason for this latitude in basal rate, we may observe that the armadillo is conspicuously chubby, presenting a low surface area compared with the long-armed and long-legged sloth. It is also relatively heavier due to its bone armor and the two species are not comparable in regard to microclimatic environment, for the armadillo with its burrowing habit does not live as exposed to the weather as does the arboreal sloth. Allee (1926) found four times less temperature variation at ground level in the jungle of Barro Colorado than in the canopy 86 feet above ground level, and it may be that arboreal mammals, on the whole, have warmer fur than the terrestrial forms in these parts of the tropics.

From our insulation and metabolism measurements it is theoretically possible to make an estimation of the critical gradient appropriate to the different animals, provided that the fur insulation is maximal and provided we can get a rough estimate of the surface area of the animal. If we know the rate of heat transfer through a given fur area at a one degree gradient, and we find that T times as much heat passes

through the same area when the fur is on the animal, then we know that the gradient is T degrees, provided that other factors remain constant.

We know the heat transfer per unit surface area and per degree from our insulation measurements and we know the total heat loss through the basal metabolism determinations; the surface can be approximated roughly by using Meeh's formula, $0.1 \times \text{Weight}^{2/3}$, which gives the approximate surface in m^2 when weight is expressed in kg. Accordingly we find the critical gradient is equal to: $\frac{\text{Cal./24H/0.1 W}^{2/3}}{\text{Cal./24H/m}^2\text{/1°C.}}$.

We see from Table III that the critical gradient, as calculated from insulation and metabolism, gives a fair correlation with the observed critical temperature of the tropical and arctic mammals. The relative order is fairly well represented and the absolute magnitudes of the gradients come out surprisingly well.

Summary

Maintenance of constant body temperature in a homoiothermic animal depends upon a balance between heat production and heat dissipation, and there are consequently three possible main avenues for climatic adaptation, (1) by body-to-air gradient, (2) by heat dissipation, and (3) by metabolic rate. There is no evidence of adaptive low body temperature in arctic mammals and birds, or high body temperature in tropical mammals and birds. The body-to-air gradient can be adapted only by means of behavioral thermoregulation (nest building, avoidance of direct sunshine, etc.). With few exceptions our adult arctic and tropical mammals and birds have a basal metabolic rate that fits the standard mouse to elephant curve, *i.e.*, the basal metabolic rate is determined by an exponential relation to size, evidently fundamental to most animals, warm-blooded or not. The basal metabolic rate is consequently not influenced by such factors as temperature gradient and insulation which largely determine the heat loss, and is hence inadaptive to climate. Equally inadaptive is the body temperature, and the phylogenetic adaptation to cold or hot climate therefore has taken place only through factors that regulate the heat dissipation, notably the fur and skin insulation.

For any temperature gradient where the body temperature is maintained, the over-all insulation and the metabolic rate must be so adjusted that their product is proportional to the gradient. This is confirmed by our material inasmuch as the observed critical gradients can be approximately predicted from fur insulation and basal metabolic rate. Under the same climatic conditions there may be an inverse relation between metabolic rate and fur insulation.

Acknowledgments

We wish to acknowledge the excellent cooperation of the officers, civilians and natives of the Point Barrow camp. We are grateful for the assistance of Captain H. W. Taylor, USN, Commanding Officer, U. S. Naval Air Station, Coco Solo, Canal Zone, in placing the facilities of the Air Station at our disposal. We wish to acknowledge the inspiration and guidance of Dr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone, during the work at the tropical laboratory, and the invaluable assistance of Mr. Walter B. Flagg and Mr. Robert H. Stapleton. We are indebted to Dr. Peter R. Morrison, University of Wisconsin, for stimulating discussion and for permission to use his weasel data. We have been fortunate in having the inspiring critical encouragement of Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton during the preparation of the data.

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REPRODUCTION OF DUDRESNAYA CRASSA HOWE[1]

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During the spring of 1949 the writer undertook, at Bermuda, certain morphological studies of marine algae designed to advance the preparation of a manuscript on the tropical Atlantic algae which he has long had in hand. Several of these studies concerned themselves with Rhodophyceae. One of these in particular, *Dudresnaya*, proved to be exceedingly easy to prepare for study, and to yield preparations in which most stages seemed diagrammatically clear; yet it proved to have a very peculiar condition in the postfertilized carpogenic branch, suggesting that perhaps further development could occur either with or without demonstrable opportunity for a diploid nucleus to enter the system of connecting filaments or oöblasts. Since tetrasporangia are not known to occur in this species, though they are well known in related genera and are the expected site of meiosis, the peculiarities of the carpogenic branch are especially interesting.

This work was facilitated by a grant from funds placed at the disposal of the Bermuda Biological Station by the American Philosophical Society. For this generous aid, and for the very cordial cooperation of the former Director of the Station, Dr. D. E. S. Brown, and of his assistant under the grant, Mr. Albert J. Bernatowicz, the writer is exceedingly grateful. During the study of this material Dr. Isabella A. Abbott (Mrs. D. P. Abbott, Jr.), who had examined specimens of this plant while monographing the family to which it belonged, made very helpful suggestions regarding the traditional account of its reproductive development.

Howe (1905) described *Dudresnaya crassa* on the basis of material which he had collected in Castle Harbor, Bermuda, in July, 1900. Our conception of the plant has not been changed materially by subsequent collections. It is one of the most striking of the Bermudian endemic algae. The plants are dull in color, becoming a little brighter and stronger rose-red on drying. The size varies up to a height of 18 cm. or a little more and, since several main branches arise near the base, clumps 25 cm. in diameter are not uncommon. Ordinarily the texture is very soft, the main branches alone being firmly gelatinous. Indeed, it is often impossible to raise the plant from the water in an ordinary dip net, for it so drains through and adheres to the meshes that it cannot be removed. While others are a little more coherent, it is seldom possible to lift a large piece from the water without breaking. The close branching is irregularly radial and alternate, long and short intermixed, without significant tendency to a bilateral arrangement. The reports of such are probably due to the effects of spreading and drying such gelatinous specimens.

The diameter of the branches varies considerably, but in general the largest are not over 5 mm. and the ultimate branches are about 1.5 mm., sometimes more. The result is that the plants seem extremely bushy and rather coarse, but exceedingly soft

[1] Contribution No. 916 from the Department of Botany and No. 159 from the Bermuda Biological Station for Research, Inc.

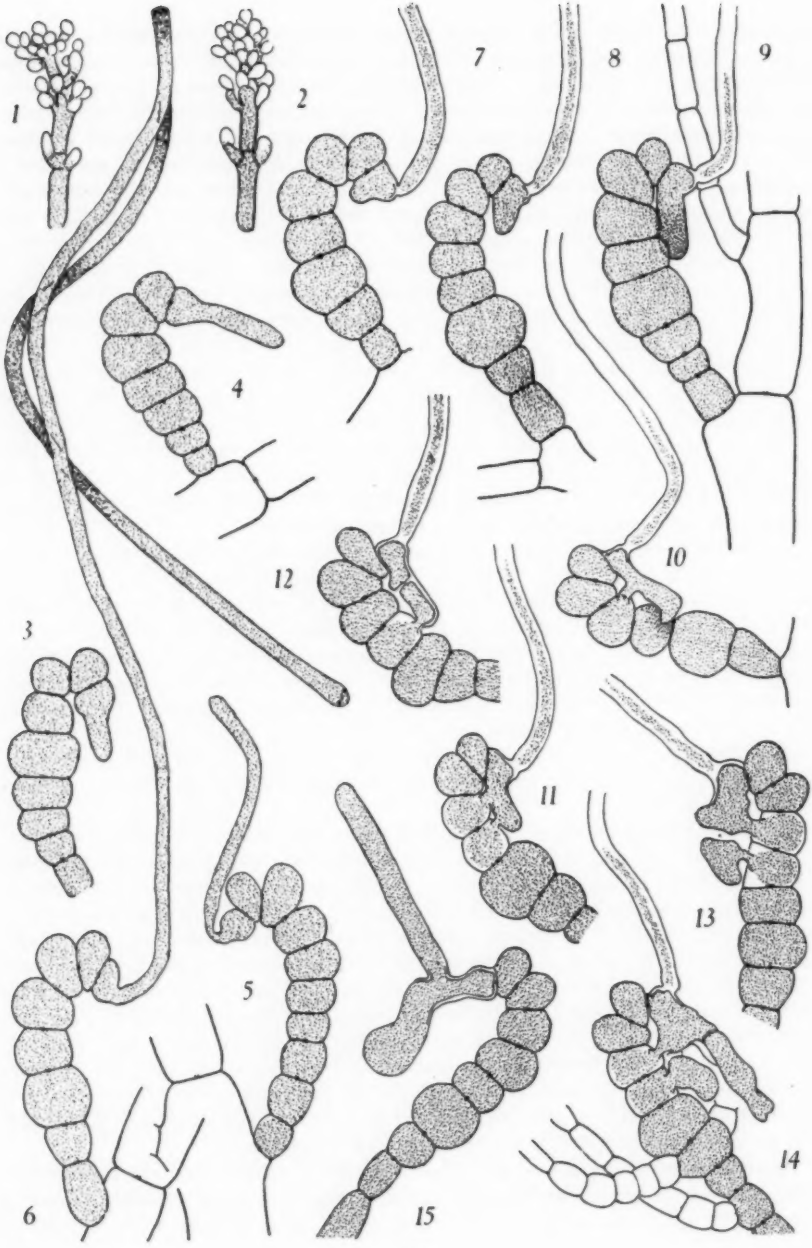
and fragile. Reproduction is commonly very abundant. Spermatangial plants are, in general, smaller and lighter in color than cystocarpic plants; the cystocarps can be seen with a 12 × hand lens. Tetrasporangia were never seen. These plants afford the most beautiful demonstrations of carpogenic and auxiliary branch development, postfertilization fusions and oöblast development and activation of auxiliary cells ever seen by the writer. They are so easy to demonstrate that the plant is marvelously adapted to classroom use. Material preserved in formalin serves adequately, though not as well as living material, but alcohol-preserved material is very poor. Staining with dilute aqueous Congo Red, followed by a little very weak KOH, gives good results on fresh or preserved material. Dried (herbarium) material can be examined for taxonomic purposes by applying a small drop of water, immediately lightly scraping off the specimen with a sharp scalpel and transferring it to a drop of the Congo Red mixture. After a very few minutes the cells will have largely returned to normal shape and the specimen may be flattened under moderate pressure, and so suitably dispersed. Occasional refractory specimens may require stronger KOH, to as much as 1 per cent, to soften them, but are rarely satisfactory.

The preferred habitat of *Dudresnaya crassa* seems to be on stones in bright, sheltered situations with moderate wave action and clear water, at a depth of 0.2–2.0 meters at low tide. It was common in the late spring at the Bermudas; in 1900 Howe secured it in July, but it disappeared in late summer in 1949.

There are about 30 Bermudian specimens, representing several collections, under this name in the herbarium of the New York Botanical Garden. Not all of these were microscopically examined, but a sampling of 12 showed all correctly named. In view of the situation respecting *D. bermudensis* in the Phycotheca Boreali-Americana of Collins, Holden and Setchell, numbers *1900* and *2196* were especially closely observed in all available collections. Number *1900* showed the auxiliary branch character beautifully, but number *2196* seemed a bit peculiar. It probably is poorly preserved, perhaps partly decayed before drying was completed. Nineteen group collections, some very large, were made between March and June in the course of the present study, from very diverse localities, but the plants were consistent in character.

Structually the plant shows a large-celled axis, the cells of the single row reaching 135–165 μ in diam., 300–430 μ in length, generally thin-walled, but becoming moderately thick-walled toward the base of the plant. This axis is reinforced by rhizoidal downgrowths which are 4.5–6.5 μ in diam., produced from the lower cells of the vegetative ramuli. On the axis cells toward the distal end there are borne whorls of four vegetative filaments which are repeatedly dichotomously and erectly branched, perhaps 5–6 times. The cells near the axis are pale, and subcylindrical with the outer end truncate by the two attached branches, but toward the distal end they carry more chromatophores and are slenderly subcylindrical with a slight swelling. The terminal cells are more tapering than the others and about 3.0–4.6 μ in diam. Thus the microscopic appearance is very different from that of *Acrosymphyton caribaeum* which is found in similar places at the same time of year, although it is doubtful if the two can generally be distinguished macroscopically.

Spermatangial plants are on the whole somewhat smaller than the carpogonial, and because of the abundance of pearly spermatangia on the surface often present a characteristic glaucous aspect in the water. The spermatangia are produced in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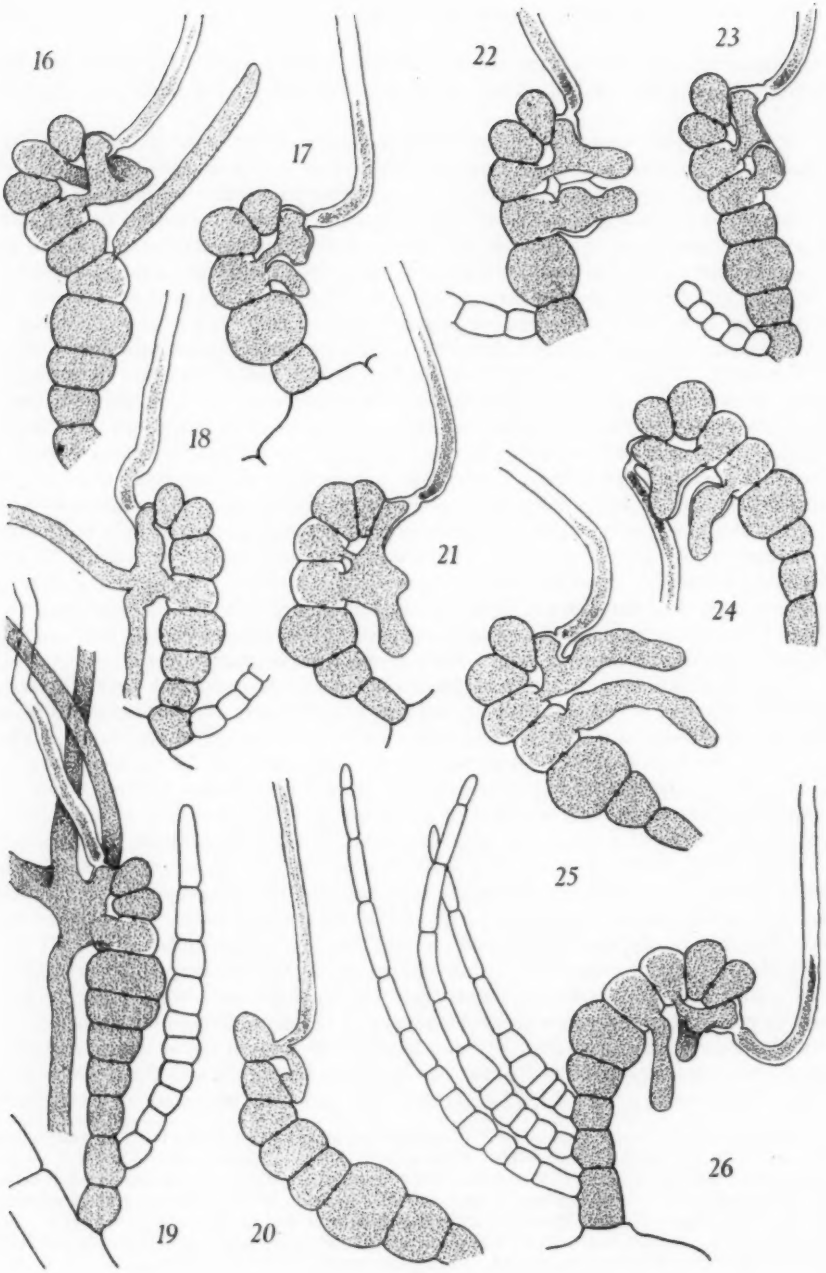
abundance on the cells toward the tips of the assimilatory or vegetative ramuli, usually opposite the stalks. They were first described by Collins and Hervey (1917).

The carpogenic branches appear laterally on the lower cells of the vegetative ramuli, alone or opposite a small branchlet, at first as a short row of a few cask-shaped cells, later reaching a length of 6–10 cells. Of these the lower may elongate a little, but the upper generally remain shorter than broad. Through the rest of this account it will be necessary to orient each observation with respect to the carpogonium, so it will be our custom to number the distal cell of the complete carpogenic branch initial, destined to become the carpogonium, as cell number 1, and those below it in succession. On this basis, then, the first distinctive change is that cells 2, 3, and 4 become wedge-shaped, causing a sharp curve, with the carpogonium rudiment close to cells 3 and 4. This rudiment then becomes flask-shaped and develops the exceedingly long, flexuous but not helical trichogyne. This, while it is at first pointed down along the carpogenic branch, quickly assumes an erect position and grows toward the surface of the plant. Mature carpogonia and many postfertilization stages were exceedingly abundant in the material available; indeed, there were so many in all stages of development that the problem was one of selection. It was quite common to find some of the lower cells of the carpogenic branch developing short simple vegetative branchlets, which generally had short cells below and more typical ones above (Figs. 14, 26, and others). The axis usually showed one cell rather larger than the others; it was usually the 5th to 7th cell from the end, but seemed to have no special function. The trichogyne often showed anomalies, particularly a simple inflation, but in some instances it forked (Figs. 15, 47). Such were probably always sterile.

After fertilization the trichogyne becomes more or less plugged near the base, leaving a remnant of cytoplasm above, and a short connecting filament is formed from the carpogonium, which proceeds to connect with a nutritive cell in the branch. The way in which this is effected does not always correspond to the classical account given for *D. verticillata* (as *D. coccinea*) by Bornet and Thuret (1876). In fact, while abundant stages and upwards of a hundred sketches recorded an ample representative series of a somewhat different story, it was with difficulty that enough examples were observed to enable preparation of illustrations to represent the expected story. To follow first the series most like this classical account, we note that the connecting filament elongates (Figs. 7–9) and makes contact with cells of the branch below it. It penetrates to first one and then another protoplast (Figs. 10, 11) by a slender tubular connection; while the proximal junction may come first (Fig. 10) it may first be complete in the distal. The carpogonium, from the beginning, retains its broad connection with the nearest point of fusion, though the trichogyne is sealed off early (Figs. 16–26). While the classical account has it that a wall cuts the connecting filament in two, so that two cells fuse separately with the two nutritive cells of the branch (Figs. 12, 23), generally no such wall appeared in the writer's

FIGURES 1–15. *Dudresnaya crassa*. Magnifications all 575 ×. Figures 1–2. Spermatangial clusters on tips of vegetative branchlets. Figures 3–6. Stages in development to maturity of the carpogonial branch. Figures 7–9. Stages in the development of the connecting filament from the carpogonium after fertilization. Figures 10–12. Junction of the connecting filament with the nutritive cells, in Figures 10 and 11 without a cross wall, and in Figure 12 with a clear one. Figures 13–14. Initiation of oöblast filaments, perhaps on each branch after activation of both cells by connecting filaments. Figure 15. Carpogenic branch with forked trichogyne.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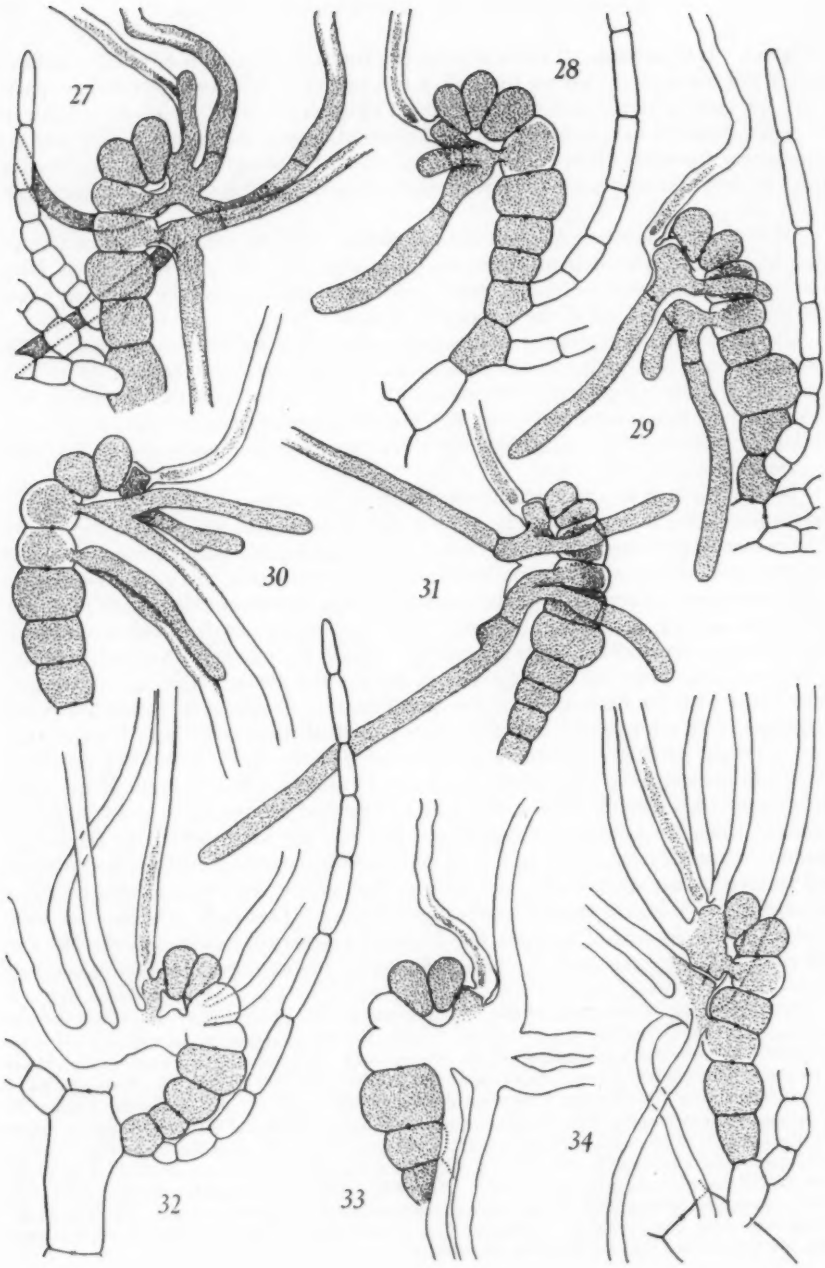
material. It is presumed that the appropriate transfer of nuclei is effected. In cases where the connecting filament fuses with two nutritive cells without previous partition, this continuity is apparently maintained (Fig. 21). Next occurs the outgrowth of separate functional oöblasts from the fusions of each of the nutritive cells with the connecting filament. It was impossible to get any thoroughly satisfactory illustrations of this, although some were reasonably convincing (Figs. 13, 14, 22, but especially 34).

Where the connecting filament did not divide, oöblasts could arise from a single fusion structure involving all elements (Fig. 21), but this was rare. It is fairly certain that the lowest cell which could produce an oöblast was the one next above the slightly enlarged cell of the carpogenic branch.

Variations in the behavior of the connecting filament were not absent. The filament could join with various cells of the branch; perhaps the 2nd (Fig. 20), certainly the 3rd (Fig. 16), most commonly the 4th, but also the 5th (Fig. 18). Some of these were anomalous (Fig. 20), but most seemed quite normal and effective fusions. Fusions with a single cell were very common, and generally yielded fully developed oöblasts (Fig. 18).

If now we turn to a second and much commoner series of events we have a different possibility to suggest. The same initial set of figures will serve (Figs. 7–9). The connecting filament fuses with a cell in the carpogenic branch, usually the 4th cell, and the carpogonium as usual retains its wide connection. It is seldom that the oöblast emerges separately from the nutritive cell in the carpogenic branch (Fig.17). Usually a swelling develops on the connecting filament opposite its point of fusion and from this the functional oöblasts arise (Figs. 18, 25, the others). Not only this, but oöblasts emerge from the next cell below, usually the 5th cell, without any evident connection with the filament from the carpogonium. Numberless examples of this were seen. In some cases it appeared quite clear that there could have been no such fusion (Figs. 26, 30). In others the proximity of the walls of the two emergent groups of oöblasts give some cause for doubt (Fig. 22). If this is the indirect development of a functional oöblast, one may assume that it was caused by a stimulation received when the adjacent or nearby cell received the outgrowth from the carpogonium in normal fashion. There is no morphological evidence that the unconnected cell receives a carpogonium-derived nucleus through the intercellular connection or otherwise. Cytological studies were not practicable. That such cells can thus form oöblasts seems confirmed by cases like Figure 16, where a fusion with the 3rd and 5th cells (skipping the 4th) has been effected by the carpogonium and connecting

FIGURES 16–26. *Dudresnaya crassa*. Magnifications all 575 ×. Figure 16. Abnormal development of a fertilized carpogenic branch. Figure 17. Development of an oöblast after communication between the carpogonium and the 4th cell only. Figure 18. Development of oöblasts after communication between the carpogonium and the 5th cell only. Figure 19. Development of four oöblasts after communication between the carpogonium and the 4th cell only. Figure 20. A carpogenic branch apparently showing a connection being established between the carpogonium and the 2nd cell. Figure 21. Development of a common fusion cell after the connecting filament had joined with cells 4 and 5, and with the initial swellings for two oöblasts evident. Figure 22. Initiation of two oöblasts from cells 4 and 5. Figure 23. Carpogenic branch with clear cross-wall in the connecting filament, which has opened communication with cells 4 and 5. Figures 24–26. Three carpogenic branches with oöblasts in early stages, coming from both cells 4 and 5 in each case, but no evidence that a connecting filament reached cell 5.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filament, while from the far-removed 7th cell (skipping the 6th) a well-developed oöblast has emerged.

Quite promptly after the first oöblasts others arise successively beside them. Several may appear from each nutritive cell; how many it is difficult to say, but cases with four oöblasts from each are not unusual (Fig. 34). The reason for this obscurity lies chiefly in the increasing transparency and indefiniteness of the whole fusion structure. Walls become thicker and more gelatinous. Cell contents become less granular and less distinguishable. Stains, as far as applied, did not give much assistance. It was clear that the junctions between the connecting filament and the nutritive cells could enlarge and the walls disappear, so that these made one broad cavity (Figs. 32, 33). The cells between the carpogonium and the nutritive cells remained strikingly distinct. Walls across the oöblasts could not be anticipated in any fixed positions; though commonly appearing a little beyond the point of origin, they usually could not be found there. Intercellular connections across these walls were noted, but rarely (Fig. 29). Walls also were noted across an oöblast behind its fusion with a true auxiliary cell (Fig. 44), but not consistently.

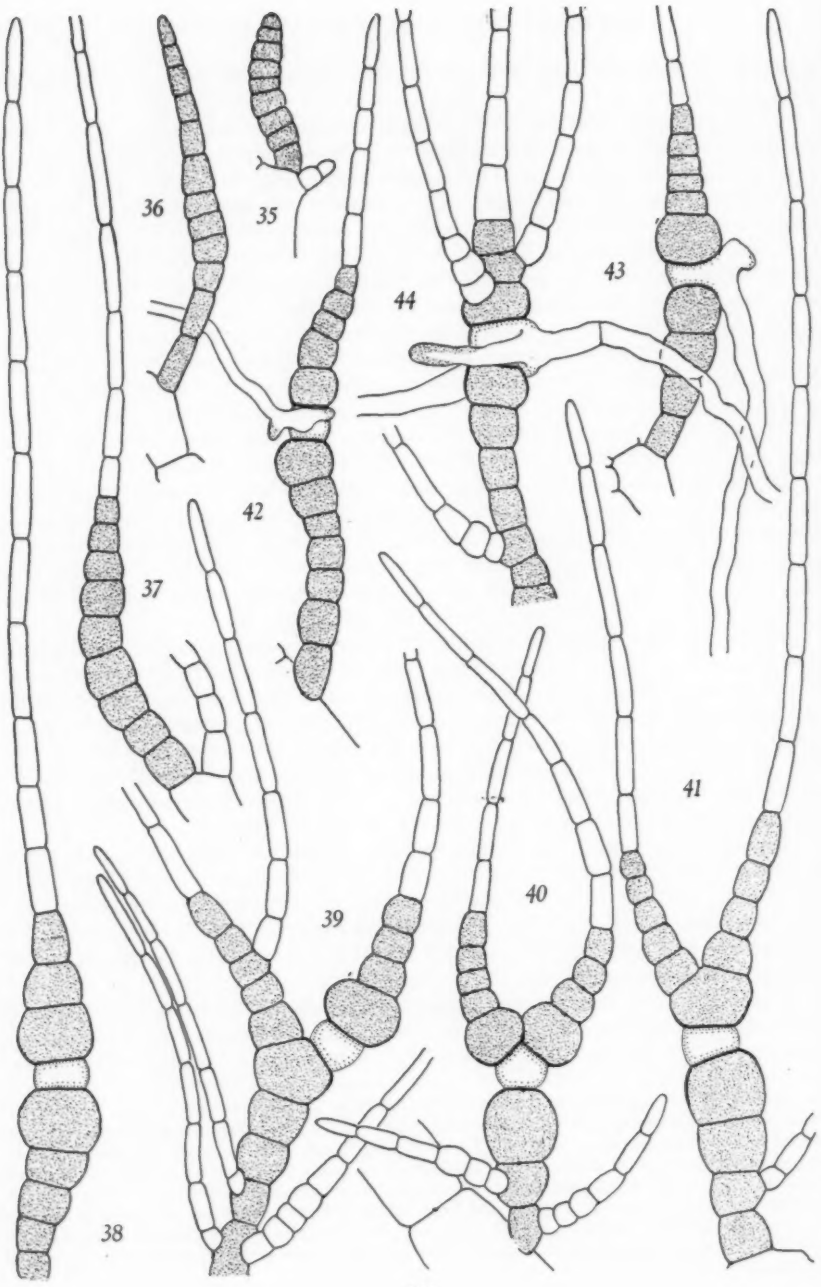
The auxiliary cells in *Dudresnaya crassa* are intercalary, in distinctive branchlets which are formed progressively during the growth of the vegetative clusters, so that several in different stages of growth may appear in one tuft. At first these branchlets are short and blunt, not unlike young carpogenic branches, but remain straight (Fig. 35). The cells near the base soon elongate, but much more so those toward the tip, producing a characteristic long piliform extension (Figs. 36, 37).

The cells toward the center, about 6–10 in number, while enlarging considerably, remain short, the length commonly less than the diameter. The final development feature is the differentiation of the auxiliary cell. This cell lags behind its immediate neighbors, which are the largest cells in the branch, appearing much smaller, rather less obviously granular in content and with a thinner wall (Figs. 38, 41). The other large cells of the branches and especially these neighbor cells seem to have an obscure, probably gelatinous thickening of the wall which shows clearly enough in stained preparations, but which is lacking on the auxiliary cell.

The auxiliary branchlet is characteristically simple, but often bears lesser lateral vegetative branchlets below, though less often above, the auxiliary cell (Figs. 39–41, 44, 48–51). Still less often, but not rarely, the axis of the auxiliary branchlet forks. This may occur at various points, and we figure examples forking on the cell below the auxiliary (Fig. 39), the cell above it (Fig. 41), and even with the auxiliary itself bearing two branches (Fig. 40).

In growing away from the carpogenic branches the oöblasts may first fuse with nearby auxiliary cells or may go some distance before opportunity for a fusion occurs. Sometimes one is favored with an exceedingly diagrammatic junction with a nearby

FIGURES 27–34. *Dudresnaya crassa*. Magnifications all 575×. Figure 27. Carpogenic branch with several oöblasts from each nutritive cell. Figure 28. Carpogenic branch with connection between carpogonium and cell 5 only, two oöblasts well established. Figures 29–31. Carpogenic branches with oöblasts well established. Figures 32, 33. Old carpogenic branches showing stages in the development of a common fusion between the carpogonium, cells 4 and 5, the remains of the connecting filament and bases of the oöblasts, which become hyaline. Figure 34. Old carpogenic branch with cells 4 and 6 acting as nutritive cells, producing eight oöblasts, their bases so approximated as to suggest that originally a connecting filament with cross-wall had been present.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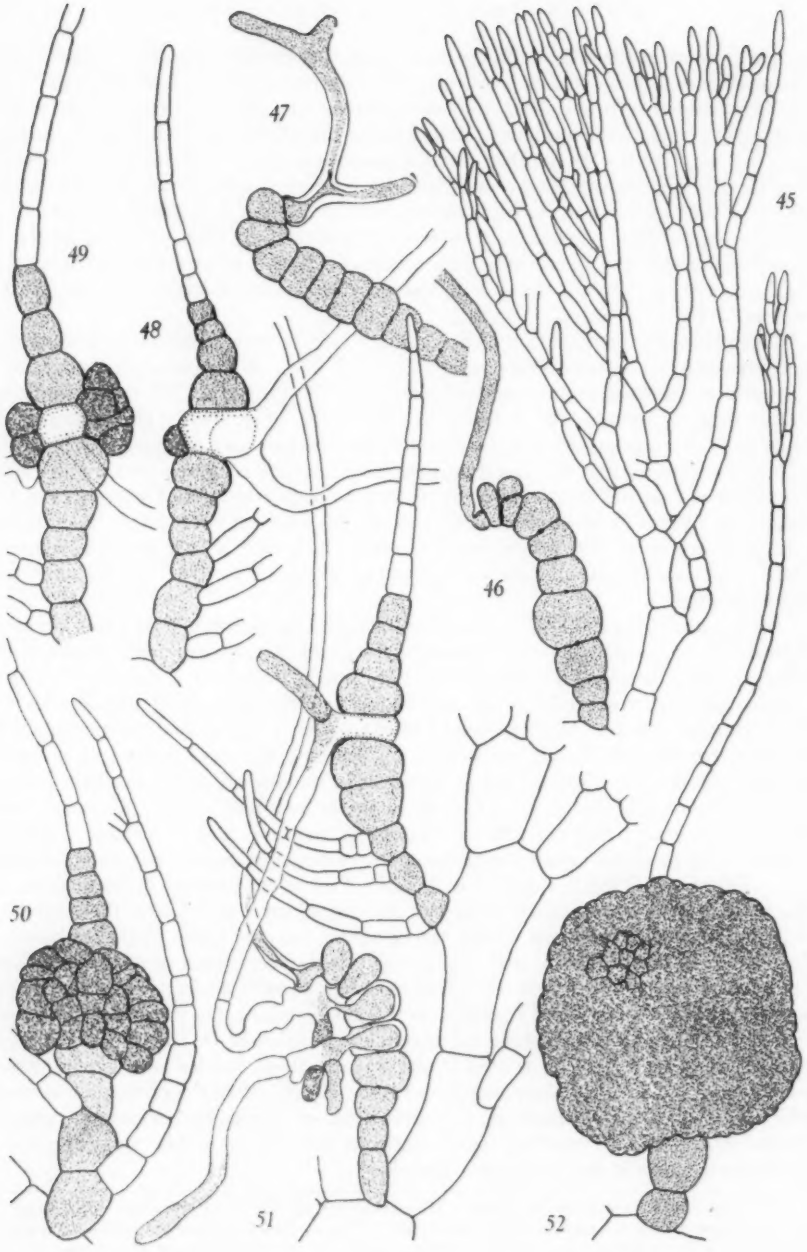
auxiliary, as when the latter is in the same branchlet cluster (Fig. 51). Sometimes it is possible to follow oöblasts from each of the nutritive cells to their respective first auxiliary fusions. In one where this occurred the distance from the 4th cell of the carpogenic branch to the auxiliary cell was 504 μ, and that from the 5th cell to its auxiliary cell was 216 μ. In another the distances were 195 μ and 357 μ, respectively, with the obvious suggestion arising that the oöblast distance from the carpogenic branch to the first point of fusion is of the order of 300 μ. It is perfectly possible to trace the oöblast from one auxiliary to the next, and this was often done, but mechanical difficulties inherent in working with material flattened by pressure preclude tracing them very far. In one that was measured, the distance from one auxiliary to the next was 396 μ.

Fusion between the oöblast and the auxiliary cells is very complete, with a general disappearance of the intervening wall (Figs. 42, 43). Immediate production of a new apex seems usual, and the new oöblast carries forward (Fig. 51). The first new oöblast may be followed by a few additional ones (Fig. 44). The cystocarp is initiated by the cutting off of a dense cell from the auxiliary obliquely opposite the oöblast connection (Fig. 48) and then, generally, a second obliquely on the other side. The result is that the round cystocarp is at first distinctly bilobed if seen from the right angle (Fig. 49), but growth soon renders this obscure (Fig. 50). The mature cystocarps are about 130–165 μ diam., short-stalked, with a long terminal filament which occasionally shows belated branching (Fig. 52). The carpospores are relatively small.

Bornet and Thuret (1867) recognized differences between their description of *Dudresnaya purpurifera* (now *Acrosymphyton*) and *Dudresnaya verticillata* (Withering) LeJolis, which they and most later writers called *D. coccinea* (C. Ag.) Crouan, but they reserved the full development of the account of that species for their beautiful "Notes Algologiques" (1876). The form of both carpogenic and auxiliary branches is clearly recognized. They saw the development of a downgrowth from the carpogonium fusing with cells in the branch, and from these fusions development of oöblasts to communicate with the auxiliary cells. As usual these are portrayed as living structures with great delicacy and accuracy. Berthold (1884) recognized the simple character of the carpogenic branch, but added nothing new. Oltmanns (1898), working with stained material, developed the nuclear account. He, even more clearly than Bornet and Thuret, recognized the formation of two cells in the extension of the carpogonium, various types of fusion between these and cells of the branch, with the later-developing communication between these and successive auxiliary cells by oöblasts. His comprehensive text (1922) has nothing new. Howe (1905) merely noted the similarity of the carpogenic and auxiliary branches in establishing the taxonomic position of *D. crassa* near *D. verticillata*. Sjöstedt (1926) restricted Dudresnaya to those species with simple carpogenic branches and intercalary auxiliary cells. Kylin (1928) confirms the account with some good new drawings. Examination of available herbarium material enabled the writer to confirm many features, but not to follow the early post-fertilization stages in the carpogenic branch.

FIGURES 35–44. *Dudresnaya crassa*. Magnifications all 500×. Figures 35–38. Stages in the development of a normal auxiliary branchlet. Figures 39–41. Three examples of types of branching auxiliary branchlets. Figures 42–44. Stages in the establishment of a connection between oöblasts and auxiliary cells, and continuation beyond the point of immediate fusion.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Bermudian material of *Dudresnaya crassa* has extended greatly our knowledge of variability in the reproductive apparatus. Both *D. verticillata* and *D. crassa* are considered dioecious, but a specimen of the former from Banyuls-sur-Mer showed numerous spermatangia on a cystocarpic plant, including a number on the branched tip of a cystocarp-bearing auxiliary branchlet. The carpogenic branches average one or two cells longer in *D. crassa* and the largest cells are not the nutritive cells but usually lie below these. Formation of a two-celled connecting filament from the carpogonium rarely seems to occur in *D. crassa* and the lower nutritive cell may develop an oöblast without direct connection. The auxiliary branchlets are longer (9–20 cells), the filiform tip is more pronounced in *D. crassa,* and the auxiliary cell itself distinctively smaller than its neighbors. The number of öoblasts, both from each nutritive cell and from each auxiliary cell fusion, seems greater in *D. crassa.* In cystocarp formation, Kylin (1928) suggests that the gonimoblast rudiments arise close to the attachment of the oöblast, but in *D. crassa* it appears that two are formed, to right and left on the opposite side of the auxiliary cell from the attachment. The cystocarp is at first bilobed, and with age seems larger and the spores smaller than in *D. verticillata.*

Tetrasporangial plants of *D. crassa* have not been found. They have been reported, though very rare, in *D. verticillata.* No nuclear changes have been reported at the time of the germination of the carpogonium which would suggest that meiosis took place at that point, and the same is true of carpospore formation. Doubt having been raised respecting meiosis at the close of a tetrasporophyte or at any other usual time, it becomes a question if fertilization can often be effective. If not, perhaps the formation of the second oöblast from the lower nutritive cell in the carpogenic branch need not be due to the receipt of any nucleus from the carpogonium: perhaps direct stimulation of a neighboring cell is enough to start it into growth. The nucleus it carries to the auxiliary cell will be haploid or diploid, depending on the state of the sexual plant, and so may be like that carried from the upper nutritive cell, in spite of not having been in communication with the carpogonium.

Summary

1. In *Dudresnaya crassa* the simple carpogenic branches reach a length of 6–10 cells. The auxiliary branches are much longer and taper at each end, but about 6–10 cells in the middle portion are enlarged, the functional auxiliary cell being a smaller one near the middle, and sterile branchlets are often formed laterally below.

2. Fusion of a spermatium with the trichogyne causes the development of a connection between the carpogonium and a lower cell in the branch. From this, and generally from the next subjacent cell, oöblasts are produced. There is generally

Figures 45–52. *Dudresnaya crassa.* Magnifications all 500 ×, except as indicated. Figure 45. Outer forkings of the vegetative or assimilatory filaments, 285 ×. Figure 46. Carpogenic branch with abnormal longitudinal division of the 3rd cell. Figure 47. Carpogenic branch with an abnormal forking trichogyne. Figures 48–50. Stages in the early development of the cystocarp. Figure 51. Unusually clear specimen showing the oöblast connection between a carpogenic branch and an auxiliary branch on the same vegetative branchlet. Figure 52. A mature cystocarp showing the persisting tip of the auxiliary filament, which in this case has branched somewhat, 240 ×.

no demonstrable connection which could deliver a diploid nucleus to the cell which produces this second oöblast.

3. The original oöblasts, and additional ones from each active cell, seem equally able to grow out, reach auxiliary cells, and initiate cystocarp formation.

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THE REPRODUCTIVE POTENTIAL OF A SINGLE CLONE OF PELMATOHYDRA OLIGACTIS

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Introduction

Very few metazoan animals reproduce asexually with sufficient rapidity and sustained repetition to render feasible a study of the theoretical asexual reproductive potential. After a culture of male *Pelmatohydra oligactis* had been maintained in a large aquarium for several years, during which it was observed that enormous increases in numbers by the process of budding occurred repeatedly, it appeared to the writer that it might be possible to determine the reproductive potential for males. The results are described in this paper.

Alternating periods of reproductive acceleration and depression were observed in the colony, the periods of acceleration coinciding roughly with abundance of food and the periods of depression to some extent with smaller quantities of food, but it was observed also that temporary declines in reproduction occurred even when food was abundant. Such a decline might have represented a general depression in the culture equivalent to senescence or a temporary resting stage. The only method of study which would yield results concerning a reproductive potential and the nature of the rhythms in reproduction appeared to be the method of isolating single specimens and following the reproductive history of the individuals and of their progeny. This method was pursued first by observing the increase in numbers in the progeny of a single specimen maintained in a mass and second, by separating the offspring from the parental animals as soon as they were detached and maintaining each in a separate culture.

Methods

Methods of culturing. A substitute for pond water was obtained by running tap water into a 50 gallon aquarium in which a quantity of the Bushy Pond Weed was maintained, and drawing off and filtering the water from time to time as needed for cultures. The water varied in pH but was always slightly alkaline. Temperatures varied from 22° to 28° C. No attempt was made to measure or to control light but the rather dim light in a corner of the laboratory was accepted as suitable since the large culture had flourished under these conditions.

The food supply was maintained at a high level. Hundreds of Entomostraca were placed daily in each culture jar. The hydra fed to capacity within a few hours and killed large numbers of animals which they did not consume. All dead food animals were removed at the end of six hours. The water in the culture jars was discarded and was replaced with fresh filtered water until the next feeding period. The high level of food supply would occur occasionally in nature but the sustained daily supply would not. Since hydra reproduced very slowly, if at all, when poorly

fed, the sustained and abundant supply was considered necessary to secure maximal reproduction.

The observations on preliminary mass cultures from single individuals were made on specimens maintained in half liter jars. Culture jars of 125 cc. capacity were used for single isolated specimens.

Methods of Recording. In a preliminary mass culture which was carried on for only 17 days all specimens were removed from the jar every 24 hours, counted and then replaced. All hydranths, including buds in all stages of development, were counted.

Isolation cultures were examined every 24 hours and separate records were kept for each specimen. Samples of a 36 day record of specimen 5.1.1 and a 33 day record

TABLE I

Reproductive record of specimen 5.1.1

Day	Serial numbers of buds detached	Number of buds attached	Serial numbers of attached buds	Number of new buds produced daily
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	2	1, 2	2
5	1	3	2, 3, 4	2
6	2	3	3, 4, 5	1
7	3, 4	2	5, 6	1
8	5	1	6	0
9	0	1	6	0
10	6	2	7, 8	2
11	7, 8	2	9, 10	2
12	9	1	10	0
13	0	1	10	0
14	10	0	0	0
15	0	1	11	1
16	11	3	12, 13, 14	3
17	12, 13, 14	3	15, 16, 17	3
18	15	3	16, 17, 18	1
19	0	3	16, 17, 18	0
20	16, 17, 18	1	19	1
21	0	1	19	0
22	19	0	0	0
23	0	1	20	1
24	0	4	20, 21, 22, 23	3
25	20, 21	4	22, 23, 24, 25	2
26	22, 23, 24	3	25, 26, 27	2
27	25, 26	1	27	0
28	0	2	27, 28	1
29	27	3	28, 29, 30	2
30	28, 29	3	30, 31, 32	2
31	30, 31	5	32, 33, 34, 35, 36	4
32	32, 33	3	34, 35, 36	0
33	34	3	35, 36, 37	1
34	35, 36	3	37, 38, 39	1
35	37, 38, 39	1	40	1
36	40	1	41	1

TABLE II

Reproductive record of specimen 5.1.40

Day	Serial numbers of buds detached	Number of buds attached	Serial numbers of attached buds	Number of new buds produced daily
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	2	1, 2	2
7	0	3	1, 2, 3	1
8	1	3	2, 3, 4	1
9	2, 3	3	4, 5, 6	2
10	4, 5	1	6	0
11	0	1	6	0
12	0	1	6	0
13	0	1	6	0
14	0	1	6	0
15	0	1	6	0
16	0	1	6	0
17	0	2	6, 7	1
18	0	3	6, 7, 8	1
19	6, 7, 8	3	9, 10, 11	3
20	9, 10, 11	2	12, 13	2
21	12, 13	4	14, 15, 16, 17	4
22	14, 15	4	16, 17, 18, 19	2
23	16, 17	4	18, 19, 20, 21	2
24	18, 19, 20	3	21, 22, 23	2
25	21, 22	2	23, 24	1
26	0	2	23, 24	0
27	23	2	24, 25	1
28	24, 25	3	26, 27, 28	3
29	26, 27	4	28, 29, 30, 31	3
30	28, 29, 30, 31	3	32, 33, 34	3
31	32, 33	2	34, 35	1
32	34, 35	1	36	1
33	0	1	36	0

of specimen 5.1.40 are shown in Tables I and II. A record was begun when a bud was detached. Thereafter the daily record for this detached bud included the appearance of new buds, the number of attached buds and serial numbers of attached buds at the time of their first appearance, and the serial numbers of these same new buds at the time of their detachment. Such a method of recording has numerous advantages. It is possible to determine by simple inspection, (1) the extent and duration of pulses of reproduction, (2) the duration of reproductive depressions, (3) the duration of the attachment periods of buds and (4) the actual rate of reproduction expressed in the production of new buds as compared to the apparent rate of reproduction which would be indicated in the numbers of attached buds. Average daily rates of reproduction were secured by considering the total number of new buds in relation to the total number of days in the period of observation. A total of 184 cultures were followed for periods ranging from 7 to 75 days and the daily average rates of reproduction were recorded.

A second and supplementary method of recording the progress of reproduction in a single clone and of numbering the individuals is shown in Figure 2. As the buds of the parent animal (5) were detached they were numbered consecutively nos. 5.1, 5.2 etc., and the numbers were placed in a clockwise series upon a surrounding circle (Gen. 1). The records of the reproduction of the detached individuals of the first generation were placed in the second circle (Gen. 2), etc. The system has the advantages of visualizing the entire output of a single animal in a single scheme, of permitting continuous expansion of the records of earlier as well as later generations and of facilitating comparisons of the outputs of members of the same generation although they are separated in time. The scheme is not intended to indicate rates of reproduction but rather extent of reproduction which may be compared in the different lines of the strain. A terminal decline in any line would be clearly indicated if it should occur. It is obvious that a high rate of reproduction

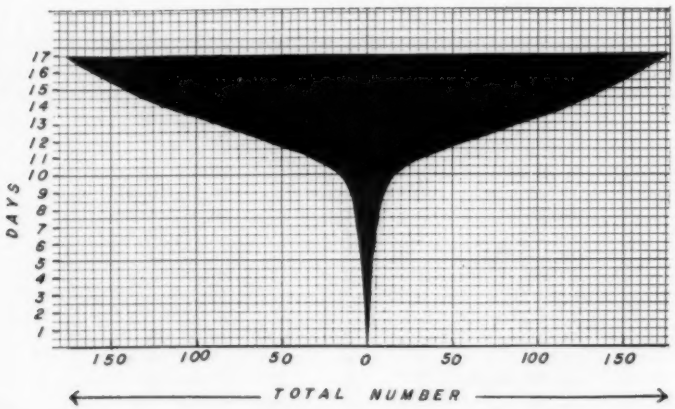

FIGURE 1. Bilateral graph showing the total number of individuals which are produced by budding by a single male specimen of *Pelmatohydra oligactis* in 17 days.

would result in such large numbers as to prohibit the culturing of all specimens and the keeping of a complete record. A complete record was kept of the first two generations but selected samples were run for the later generations. Approximately 3500 specimens from 184 cultures were recorded upon the scheme shown in Figure 2. Included in the 184 cultures were 15 cultures in which the reproducing individuals died during physiological depression.

OBSERVATIONS

Single clone mass culture. The specimen which produced the mass culture shown in Figure 1 was selected from a large tank in which hydra had been maintained for several years. The specimen was large, free from gonads and buds and was in a healthy condition. The results of culturing this individual along with its progeny with an optimal food supply are shown graphically in Figure 1. Single budding

occurred within 2 days and by the end of the eighth day some of the specimens in the culture were bearing one bud, some were single and a few were bearing 2 buds. Specimens bearing 4, 5 and 6 buds appeared rapidly during the next 2 days but on the last day of the 17 day period there was an increase in the number of single specimens which were not budding. Increase in numbers was very high from the tenth to the fourteenth day but the rate of increase declined slightly from the fourteenth to the seventeenth day when the culture was discontinued. The production of

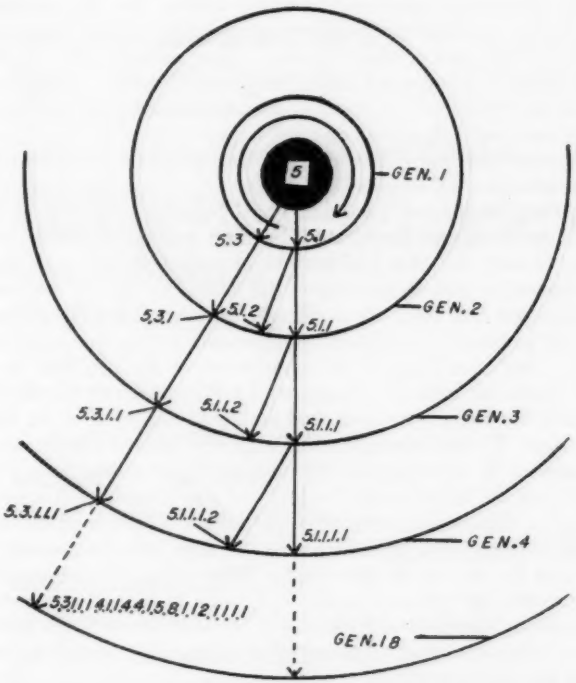

FIGURE 2. Scheme for recording the population produced by a single hydra. The scheme is capable of recording a continually expanding population and of indicating the relations of specimens by generations. The scheme does not express relations in time.

350 specimens in a period of 17 days from a single specimen was a remarkable performance which was repeated by other specimens cultured under the same conditions. One of the cultures maintained for 22 days was marked by a steady decrease from the maximal rate of reproduction after the thirteenth day. The beginning of a decline in reproductive rate was not a result of an accumulation of decomposition products in the culture water since the water was changed daily. Neither was it due to crowding or a lack of food or oxygen. It was concluded from this preliminary set of observations that some internal physiological state in the strain was responsible for

the reduction in reproductive rate but that the situation could be studied better in pedigreed individuals rather than in masses, and consequently the method of isolating single specimens as soon as they were detached was substituted for mass culturing.

Reproduction in isolated cultures. Prolonged observations upon isolated cultures were made with the primary objectives of determining the pattern in reproductive rate and of discovering whether a clone maintained under the conditions described would continue to reproduce indefinitely or would become senescent and die out. Many correlated facts were secured during the observations. It was observed that during maximal pulses of reproduction new buds might be produced by an individual at intervals of about 5 hours. The minimal time for the development and detachment of a bud was found to be about 17 hours. Multiple buds upon a parent animal are the result, in part, of the differences in time of the short intervals between new bud formations and the longer interval between the formation and the detachment of a bud. The time interval between the formation of buds is greatly extended in periods of poor food supply and also in periods of physiological depression. During periods of profound physiological depression or in complete absence of food no buds are formed. The time necessary for the growth, differentiation and detachment of a bud may be extended greatly. During the early stages of development a bud depends upon the parent animal for sources of energy but in later stages the bud feeds for itself and becomes relatively independent. If the parent animal becomes physiologically depressed during the early stages of the development of a bud, the growth and detachment of the bud may be postponed. An instance is shown in Table II (specimen 5.1.40, column 4) in which bud no. 6 arose on the tenth day but was not detached until the conclusion of the depression on the eighteenth day. If the depression in the parent animal occurs when a bud is in the later stages of its development the bud will become detached. The interval between the time of the detachment of a bud and the formation of a new bud upon the detached bud is subject also to extreme variation. When maximal reproduction is occurring the tendency to rapid formation of new buds is present in both the parent animal and the first of the new buds. Buds have been observed frequently to form buds of their own before they are detached. However, a bud detached in a period of approaching depression in the parent animal will not form buds of its own immediately and if the food supply is low the new bud will postpone its own budding until conditions are more favorable.

An examination of Tables I and II brings out the fact that there is a general correlation between the number of buds detached per day and the number of buds attached to the parent at about the same time. There is also a general correlation between the number of buds attached to the parent animal at one time and the production of new buds at the same time. The occurrence of several buds upon a parent animal is an indication of a period of rapid reproduction but it cannot be taken as an exact index of reproductive rate. For example, in Table I it is indicated that on the thirty-first day 5 attached buds were present on specimen 5.1.1 and the number of new buds formed on that day was 4. On the thirty-second day 3 attached buds were present but no new buds were formed on that day. In Table II it is shown that a bud (no. 6) was present upon the parent animal from the ninth to the eighteenth day. A casual observation of the fact would indicate that the parent

animal was reproducing at a low rate when, in fact the parent animal was in a state of depression and did not form a new bud for seven days. Because of these apparent discrepancies between the number of buds attached or detached on any one day and the number of new buds formed on the same day, the rate of new bud formation was selected as the best criterion of an index for reproduction rate.

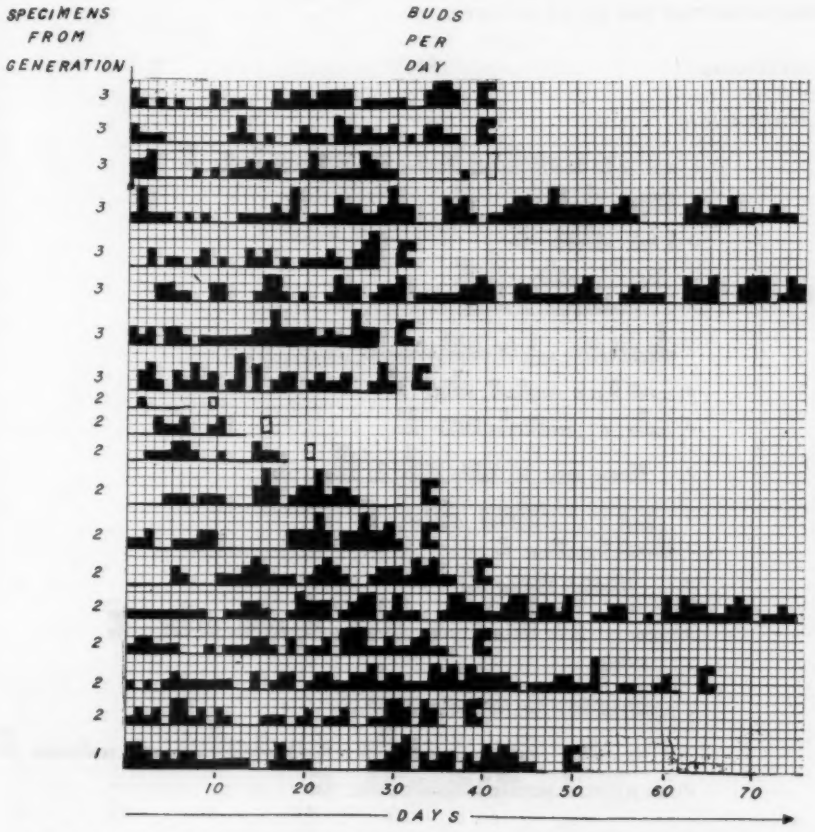

FIGURE 3. Block graph showing the daily production of buds by the single parent hydra and 18 specimens of the second and third generation. The symbol ⊏ indicates that the strain was terminated intentionally or was lost accidentally. The symbol □ indicates that the specimen died during a depression.

The rate of new buds produced per day by the original member of clone 5 and of 183 bud descendants representing samples from 18 generations has been plotted and 52 typical samples are shown in Figures 3, 4 and 5. Records followed by the symbol ⊏ are those which were intentionally terminated or were lost by accident. The 5 records followed by hollow rectangles indicate lines which died during physio-

logical depression. The average rate of reproduction for each generation and for all individuals cultured is shown in Table III. The average daily production of new buds per day for all individuals cultured was 1.14 (Table III). The inclusion in the fifth and sixth generations of several cultures which died during depressions is responsible for the low rates per day shown in the Table. The reproductive rates of specimens which eventually do not survive a depression is considerably lower than the average rate for all specimens.

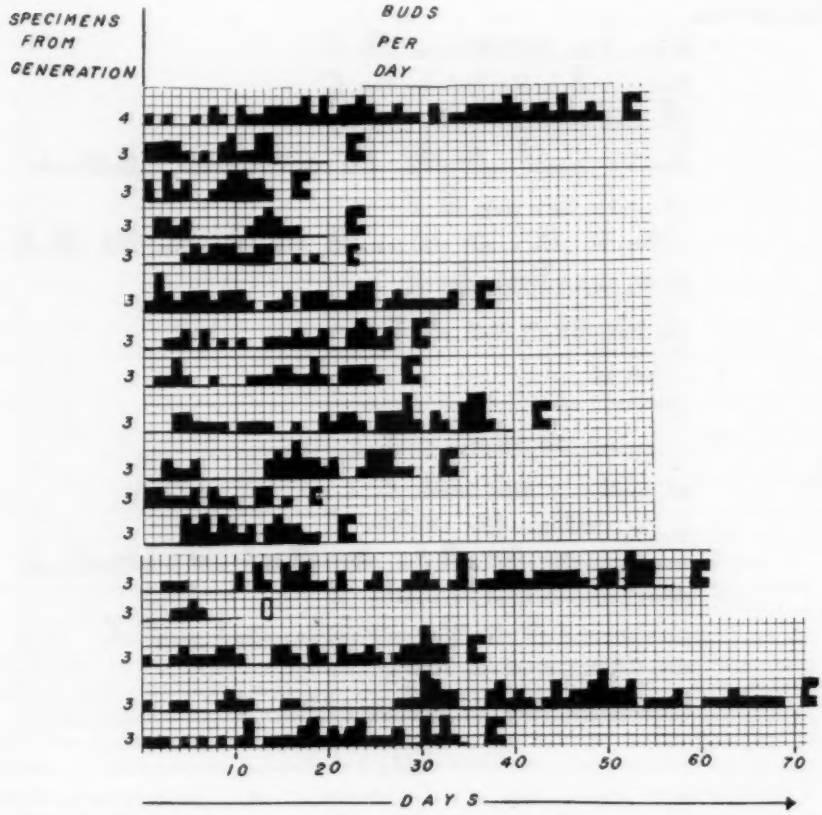

FIGURE 4. Block graph of 17 specimens from the third and fourth generations.

An analysis of reproduction and reproductive rates as illustrated in the graphs and Tables I and II reveals the following facts: (1) Reproduction occurs in rhythms characterized by periods of acceleration and shorter periods of rest or lowered rates per day, (2) There is no sustained reproduction at the highest level. The highest rate of reproduction for any one day is 5 and this rate is attained only twice. The longest period of sustained production of 4 or more buds per day is 2 days and 22

such periods are shown on the graphs. The longest period during which, on consecutive days, 3 or more buds are produced per day is 3 days. Eleven days marks the longest continuous period for the production of 2 or more buds per day and in no instance is there a period of more than 24 consecutive days in which one or more buds are produced. (3) In addition to the one or two day interruptions which oc-

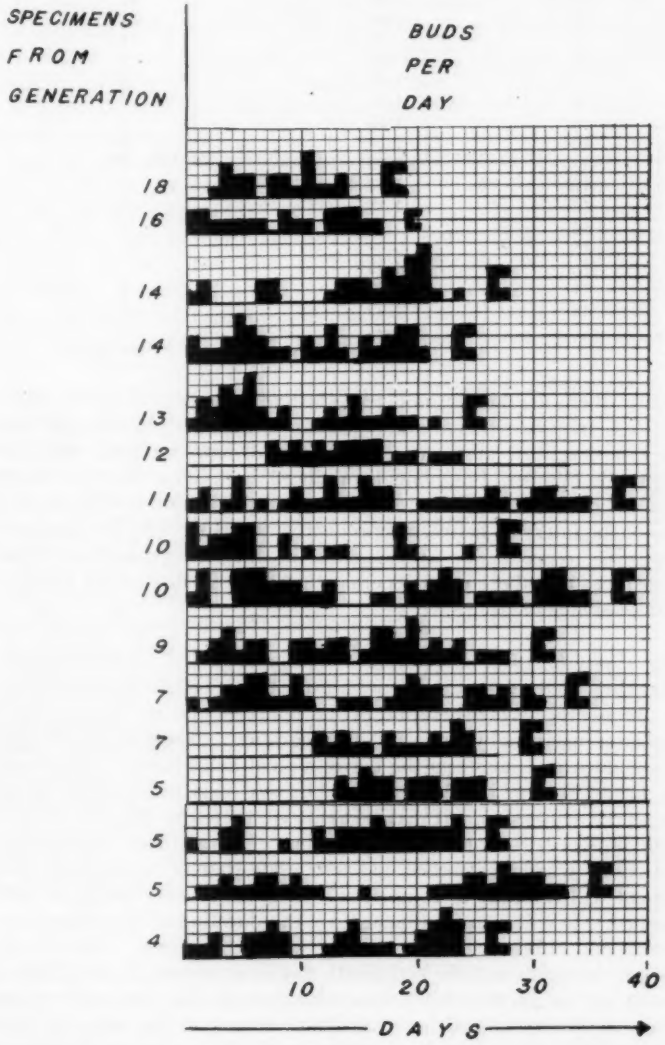

FIGURE 5. Block graph of 16 specimens from the fourth to the eighteen generations.

TABLE III

Rate of reproduction as indicated by production of new buds per day in all cultures

Generation	Average of new buds per day	Number of cultures
1	.96	22
2	1.05	50
3	1.10	42
4	1.04	22
5	.78	14
6	.65	8
7	1.00	3
8	1.15	3
9	1.18	3
10	1.27	3
11	1.05	3
12	1.31	2
13	1.43	2
14	1.24	2
15	—	—
16	1.43	2
17	1.75	2
18	1.67	1
	1.14 Gen. Av.	184 Total

cur frequently there are longer, non-reproductive periods of 3 to 12 days. These longer and less frequent intervals are periods of genuine physiological depression and they will be described later. (4) The pattern of reproduction in each individual, while irregular in number of individuals produced, is rather uniform in its repetition. There is no indication of a decline in the rate of reproduction per day or increase in the lengths of the intervals between reproductive periods. (5) No marked changes in the pattern of reproduction are apparent in different generations, from which it may be deduced that there is no general or progressive diminution in reproductive vigor in successive generations.

Periods of physiological depression. The longer periods of 3 to 12 days during which no budding occurs are marked not only by a cessation of reproduction but also by inactivity, frequently by degeneration of the tentacles and the hypostome and in a lesser number of instances by death and disintegration. The onset of this physiological state is sudden and it affects the entire animal immediately. The onset of the state is so abrupt in some instances that one or two buds still attached to the parent are caught in the depression. It may be assumed, therefore, that the onset occurred between the time of bud formation and the time of normal bud detachment. If the depression occurs while an attached bud is in a late state of development the process of detachment will continue to completion. If a bud is very young it will not become detached. It will remain attached for the duration of the depression during which growth and differentiation in the bud will not progress. The history of bud no. 6 between the ninth and the eighteenth day in specimen 5.1.40 (Table II) is a case in point. It is inferred from these observations that the state of depression originates in the parent animal but that it may extend to the bud. If the bud has established a sufficient degree of independence it will not be much affected by the

depression in the parent animal. The fact that young buds do not grow and develop during depressions indicates that the state of depression extends to the processes of growth and differentiation as well as activity and reproduction.

The state of depression is set off sharply from the state which exists in partial starvation. If food is provided for a partially starved hydra the hydra reacts immediately and vigorously. If the food supply is meager the animal will produce buds slowly. If the food supply is abundant it will produce buds at an accelerated rate. On the other hand, animals in the depressed state react slowly to tactile stimulation and do not react to food at all. Cladocera, which would be captured immediately by a vigorous hydra, collide with the depressed hydra and evoke no reaction in the tentacles or nematocysts although they frequently come into contact with the tentacles and sometimes rest upon them. The starved animal possesses a full capacity for vigorous reaction, growth and reproduction while the depressed animal is incapable of vigorous reaction or growth or reproduction and in deep depression the most differentiated structures disintegrate.

Recovery from a depression is marked by a resumption of activity and feeding and, on the part of the animals that have not undergone partial disintegration, a prompt resumption of budding if the food supply is abundant. The animals which have partially disintegrated regenerate the lost parts and frequently exhibit a partial loss of morphogenetic control. The regenerated apical region is sometimes double and instances of development of supernumerary tentacles are common. In a few rare instances buds, which remained attached to the parent during the depression and were depressed themselves, on recovery from the depression remain attached to the parent but acquire sufficient independence to form a colony with the parent. Structural abnormalities which arise during depression and recovery are slowly corrected by a process of regulation. The remarkable adjustments which occur during the process of regulation will be made the subject of a separate paper. The length of a period of depression is not necessarily a mark of its severity. A depression period of 8 days may be characterized by nothing more severe than a loss of activity and reproductive output while a 3 day depression in another specimen may be accompanied by disintegration of tentacles and hypostome.

The character of the depression aside from the affects described above is not known. It might be postulated that there is a loss during rapid reproduction of some substance essential for normal metabolism, but the fact that the animals recover from the depressions and regenerate lost parts without feeding indicates rather a disturbance of arrangement of substances or processes prior to the depression and a rearrangement of the materials or processes during depression. Recovery of capacity to react to food and to metabolize the new intake would necessarily precede the actual capture and utilization of food. With the resumption of feeding and metabolizing of food a store of energy would be furnished, a part of which could be diverted to a resumption of the process of budding.

Reproductive Potential

A satisfactory formula for the theoretical reproductive potential of an animal should be capable of expressing a reproductive output for a stated period and be capable also of unlimited expansion if the factors which control reproduction are invariable. Such a formula is easy to construct in the case of a sexually reproducing

animal which has a limited life span and undergoes senescence and death on the part of the parent animal. Only the progeny need be considered as the reproducers of the future. In the sexually reproducing animal the number of viable gametes, the numbers of new generations per year or season and the span of time during which the parent is able to reproduce are most important. The situation is different in an animal which reproduces asexually and in which both parent and offspring survive to reproduce on equal terms. The parent reproduces continually and long spans of time elapse between the production of the first bud and a much later one. All of the buds from a single individual belong to the same generation but the origin of the members of the single generation is spread through a long span of time. The continuity of reproduction in all generations at the same time makes it impossible to estimate the total output which constitutes one generation, or to separate the generations in time. A different method of approach must be employed if the total theoretical production is to be expressed in terms of time.

When hydra produces a mature individual by budding, and both the bud and the parent survive and continue to bud, a mathematical doubling occurs each time a bud is produced. With continual budding the doubling increases exponentially. If a rate of budding (doubling) can be established this rate can be used as the exponent of 2. If the rate is expressed in days and the daily average rate is known the following formula can be used for any stated length of time:

Reproductive output for n days $= 2^n \times$ average daily rate.

The only factor which might vary would be the average daily rate of doubling. Reproduction in the original member of clone 5 and in the samples of its progeny has many minor fluctuations and long periods of depression, and some strains die out during depressions. However, an average daily rate of bud production has been obtained by including these depressed strains among the 184 samples. The records of the specimens which became depressed and eventually died have been included because each specimen was an actual or potential reproducer as long as it was alive. It has been shown also that, within the limit of 75 days at least, there is a general continuity of the daily rate in the over-all performances of individuals and of different generations. The daily average has been shown to be 1.14 under the favorable conditions described. Using 75 days as the period of time during which it is desired to express a total output, the total output under the conditions described can be expressed as follows:

$$\text{Reproductive output for 75 days} = 2^{75} \times 1.14.$$

The possibility that the potential indicated above for 75 days applies in longer periods was tested tentatively in the following procedure. Three of the oldest individuals reported for the 75 day period were observed for 48 days longer before they were accidentally lost. The average number of buds produced by these individuals for the total period of 123 days was 136. The average daily bud production was 1.10. Also two cultures of the most recently produced individuals from the 75 day period were maintained for an additional period to determine whether the production of succeeding generations would decline. The number of generations was raised to 28 in one culture and 26 in the other before they were lost accidentally. If these results are added to those described for the 75 day period it appears that there is no decline in the average daily rate of bud production or in the capacity of new buds

to produce new generations, and that the formula for the total output can be used to cover a period of at least 123 days.

Discussion

Tremendous hydra populations have often been noted in natural uncontrolled situations. Some of these populations have been noted as to quantity and date with no observations as to duration. Fishing nets in Lake Erie, (Clemens, 1922) Lake Michigan (Welch and Loomis, 1924) and Lake Superior have been described as covered with hydra, in some cases for thousands of feet. Immense populations have been observed in the inland lakes of New York and Wisconsin. In some instances the inlets of water systems have been clogged with hydra. The duration of a large population in Lake Douglas, Michigan has been studied by Welch and Loomis. The large population existed during late spring and early summer but declined in late summer after the water temperature rose permanently to a point above 70° F. Large populations have been observed in other cases in late July, in November and in January, February and March in natural habitats with low temperatures at other seasons. It is indicated by these observations of hydra in natural habitats that accelerated reproduction is not seasonal except as seasonal conditions are responsible for low temperatures, food supply and dissolved oxygen in the water. In a large tank in which favorable conditions have been maintained throughout the year, the writer has observed reproductive pulses resulting in the production of thousands of specimens in each month of the year. It is clear that hydra may enter a period of reproductive acceleration at any time but, as indicated in the graphs, the periods of acceleration are sure to be followed by periods of depression. Under natural conditions a few hydra, which have survived unfavorable conditions and are in a semi-starved state, will form an enormous population within two or three weeks when favorable conditions exist. If favorable conditions prevail for several weeks the extent of the population will be limited, for the most part, by the death of specimens which become depressed. During the depression specimens do not move about and the ability to undertake vertical migrations into cooler water is lost. These specimens do not feed during the depressed period and if the food supply should decline in the immediate vicinity during the depression the animals would be in an unfavorable situation on their recovery. It seems, therefore, that great populations are reduced in part by extrinsic unfavorable factors in the environment but that an important contributing factor is the intrinsic depression which inevitably follows any initial burst of reproductive acceleration.

The phenomenon of depression was first noted by Trembley in 1744 and has been studied by numerous investigators (R. Hertwig, 1906; Frischholz, 1909; Rehm, 1925; Goetsch, 1922; Grosz, 1925). Hyman (1928) has made an extensive study of the metabolic rate during depression, recovery from depression and starvation. She agrees with earlier investigators that depression may be induced by long continued feeding, extremes of temperature, insufficient oxygen and changes from pond water to tap water, but she comes to the conclusion that the primary cause is the condition of senescence during which there is a general lowering of the rate of metabolism. It follows that recovery from the depression, marked by a regeneration of lost parts and a resumption of budding, indicates a process of rejuvenescence. In none of the

studies mentioned above has accelerated reproduction been emphasized as a causative factor for the depression. In the observations described by the writer depression has occurred only at the end of a period of rapid budding. It would appear that some substance necessary for normal metabolism is exhausted by rapid budding or that some toxic substance is formed incident to the rapid budding and that the lowered rate of metabolism may be caused thereby.

Senescence in highly differentiated animals is terminal and results in death. If the process of depression is a process of senescence in hydra, it is terminal for a relatively small number of specimens. The others, after partial senescence, undergo rejuvenescence and the specimens survive and resume reproduction by budding. It appears that in hydra partial senescence has been substituted for terminal senescence for most specimens and that, given favorable conditions for recovery and reproduction after depression, reproduction by budding can go on indefinitely.

Summary

1. A single *Pelmatohydra oligactis*, when maintained under favorable conditions of temperature and water and given maximal amounts of food, reproduces rapidly and continually. New buds are formed every five hours when reproduction is at its height and buds may be detached in 17 hours after their first appearance.

2. Periods of maximal reproduction alternate with periods of depression during which specimens become inactive and in many instances degenerate at the apical ends. Some specimens die during the periods of depression but most of them regenerate the lost parts and resume reproduction by budding.

3. The average daily rate of bud production in 184 specimens, including those which eventually died during depressions, was 1.14.

4. There is no general decline, during a 75 day period, in reproductive vigor and rate in single specimens or in succeeding generations. During the 75 day period 18 generations were produced.

5. The total potential production by a single animal for a 75 day period can be obtained by using the average total number of doubling (budding) for the period, 75×1.14, as an exponent of 2.

6. Sudden reductions in population may be directly caused in natural habitats by unfavorable environmental factors, but the physiological depressions which follow rapid reproduction contribute to the decline by rendering the animals inactive and unable to accommodate themselves to changing conditions.

7. The depressions which follow rapid reproduction are basic intrinsic physiological states which may be genuine states of senescence. They may be caused by exhaustion of some substance essential to normal metabolism or to the accumulation of some substance which interferes with metabolism. Specimens appear capable of repeating the cycles of reproduction and depression indefinitely under favorable circumstances.

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OSCILLOSCOPIC AND STROBOSCOPIC ANALYSIS OF THE FLIGHT SOUNDS OF DROSOPHILA [1]

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The problem of the flight sounds of insects has recently been brought to focus in the admirable monograph of Sotavalta (1947). According to this author it was in 1866 that Mühlhäuser first suggested that "the pitch of the flight-tone was exclusively caused by the number of wing-strokes per second." By matching the tone of the flight sound with that of a suitable tuning fork, one should then be in a position to assign a corresponding frequency to the wingbeat itself. This possibility was exploited by Landois (1866) and became standard practice. Unfortunately, such a procedure is complicated by various difficulties, among which is the "sopranotenor error" whereby the flight tone is matched with a tuning fork having twice the proper frequency (Sotavalta, 1947).

Sotavalta has revived the acoustic method with notable success. Endowed with "absolute pitch," this investigator has been able to dispense with apparatus and, by the direct use of his ears and musical training, to appraise the frequency of wingbeat of free-flying insects. Thus the flight sound of insects has become a matter of contemporary interest as a quantitative approach to the study of insect flight.

It is therefore surprising that the literature contains no adequate analysis of the flight sound of any insect or definite proof that acoustic studies record the same frequency of wingbeat as do more objective methods. As Chadwick (1939) has pointed out, "the relation between the pitch of an insect's tone and the frequency of its wing-motion is still not completely understood."

Solution of this problem depends on methods whereby measurements of both the flight sound and the frequency of wingbeat may be accomplished simultaneously on the same flying insect. This objective is well within reach of present methods. The electronic stroboscope, first applied by Chadwick (1939) to the study of insect flight, permits prompt and accurate measurements of the frequency of wingbeat. Concerning the flight tone itself, Davis and Fraenkel (1940) mention its measurement by the use of a microphone and cathode ray oscilloscope, and some preliminary application of this technique has also been described by Sotavalta (1947).

In the present investigation we have applied both the stroboscopic and oscilloscopic techniques to flying insects in an attempt to establish a direct correlation between the flight sound and the frequency of wingbeat.

[1]The research here reported was carried out at the Psycho-Acoustic Laboratory under contract with the Office of Naval Research, U. S. Navy (Contract N5ori-76, Project NR142-201, Report PNR-96), and was aided by the Lalor Foundation of Wilmington, Delaware. Reproduction for any purpose is permitted by the U. S. Government.

Material and Methods

Our first experiments were performed nine years ago on the fruitfly, *Drosophila funebris*, and the mosquito, *Culex pipiens*. Although the primary problems were solved at that time, the study has been recently repeated with improved techniques of sound recording. These latter studies have been performed solely on *Drosophila funebris*.

In brief, the tip of the abdomen of each fly was attached with melted paraffin to a small wire, according to a method described previously (Williams and Chadwick, 1943). The insect was then oriented 1.5 cm. in front of a Western Electric 640 AA condenser microphone. This assembly was located in a sound-proof room from which leads passed to an adjacent room housing the recording apparatus. The latter consisted of an oscilloscope of linear sweep characteristics used in conjunction with a preamplifier and a camera of stable, adjustable film speed. A check on film speed was provided by a neon light, placed in the field of the camera and driven by a half-rectified AC potential from an audio-oscillator. The recording system had an essentially flat response from a few cycles to 5000 cycles per second.

In addition to the insect, microphone, and switch for remote control of the camera, the first room also contained a General Radio "Strobotac" for independent measurement of wingbeat frequency by the stroboscopic method. In practice it was found that the noisy discharge of the stroboscopic tube interfered with the sound picture. In order to correlate the two types of measurement, the following procedure was adopted.

Flight was induced by a puff of air. The insect's performance was tracked with the stroboscope until the animal showed constant frequency of wingbeat. The stroboscope was then switched off, the camera started, and the sound picture recorded either on continuously moving film or in a number of consecutive sweeps of the oscilloscope. Flight was terminated by touching the animal's feet with a steel rod.

For the purpose of recording the sound picture from the outset to the termination of individual flights, stroboscopic measurements were discontinued, and the flight sounds recorded on continuously moving film.

Relation of Flight Sound to Wingbeat Frequency

Unlike the sound of a tuning fork, the buzz of a flying insect is by no means a pure tone. As shown in Figure 1, the exact character of the sound picture depends on the orientation of the insect with respect to the microphone. Though a sinusoidal waveform is never encountered, each orientation of the microphone yields a definite repeating pattern of fundamental wavelength.

In Table I wingbeat frequency has been estimated from the records in Figure 1 on the assumption that each repeating pattern corresponds to one cycle of wingbeat. The validity of this assumption is demonstrated by the excellent agreement between wingbeat frequencies in each flight as measured by oscilloscopic and stroboscopic techniques. This agreement persists when wingbeat frequency is increased by clipping the distal third from the tips of the wings.

These observations afford the first definite proof that, within the limits of experimental error, the fundamental frequency in the flight sound is the same as the frequency of wingbeat.

Details of the Sound Picture

As mentioned in the preceding section, the flight sound presents a waveform that depends on the orientation of the insect with respect to the microphone. In our oscilloscopic records, samples of which are illustrated in Figures 1 and 4, compression of air at the microphone is recorded as a downward deflection of the beam and rarefaction as an upward deflection. With the microphone anterior to the longi-

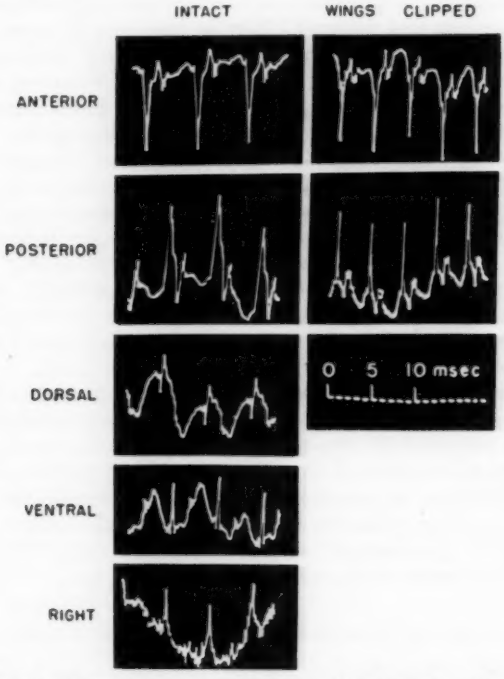

Figure 1. Sound produced by *Drosophila* during "fixed" flight. The orientation of the microphone with respect to the animal is stated at the left of each record. Compression of air at the microphone is recorded as a downward excursion. The records in the right hand column were obtained after clipping approximately one-third from the tips of the wings. Clipping the wings is seen to have little effect on the sound pattern despite the marked increase in wingbeat frequency.

tudinal axis of the insect, it will be observed in Figure 1 that the sound pattern is almost exactly the inverse of that recorded with the microphone posterior to the insect.

As judged by its position when the insect is not flying, the baseline of zero change in air-pressure seems to lie approximately midway the vertical excursion of the oscilloscopic beam. Thus, as diagrammed in Figure 2, the microphone records a predominantly upward excursion or rarefaction anterior to the insect and a predomi-

TABLE I

Wingbeat frequency of Drosophila determined by oscilloscopic and stroboscopic techniques

	Sound frequency (beats/second)	Stroboscopic frequency (beats/second)
Intact insect:		
Head toward microphone	166	164
Abdominal end toward microphone	182	180
Dorsal side toward microphone	182	183
Ventral side toward microphone	182	186
Right side toward microphone	193	194
Wings clipped:		
Head toward microphone	250	247
Abdominal end toward microphone	270	261

nantly downward excursion or compression posteriorly. In terms of air flow this signals a net, polarized movement of air from front to rear (Demoll, 1918; Magnan, 1934).

Figure 2 attempts to correlate these pressure changes with the cycle of wingbeat. Whereas the latter is approximately equally divided into upbeat and downbeat (Magnan, 1934), the cycle of air-pressure is decidedly unsymmetrical. According to the analysis diagrammed in Figure 2, anterior rarefaction and posterior compression are present during approximately 85 per cent of each cycle of wingbeat. Only a small fraction of the wingbeat cycle, indicated by the spike in the records, produces anterior compression and posterior rarefaction. It seems necessary to conclude that polarized flow of air from front to rear is generated, not only by the downbeat of the wings, but also by a significant proportion of the upbeat.

Though the large and transient "spike" in each cycle of wingbeat cannot be accurately localized with the present data, we suspect that it corresponds to the

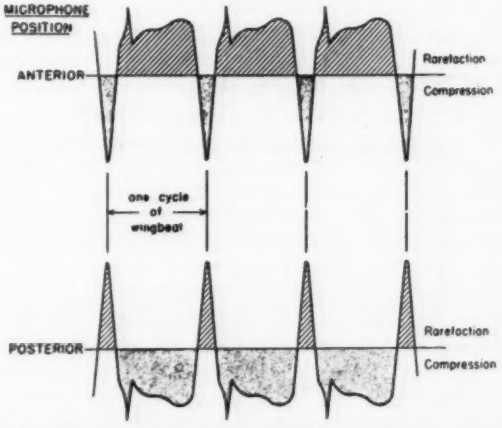

FIGURE 2. Correlation between the flight sound and the wingbeat. One cycle of sound corresponds to one cycle of wingbeat. For further explanation see text.

movement of the wings through the upper limits of their trajectory. This point could presumably be settled by studies in which the sound picture and the position of the wings were recorded simultaneously. Further study is also required to interpret the components of low amplitude that complicate the sound picture; especially is this true in regard to the rapid biphasic wave interpolated in each cycle of wingbeat (Fig. 2). Turbulences, movements of the thorax itself, and vibration of the halteres (at the same frequency but in opposite phase to that of the wings) are among the possible sources of such minor components.

Frequency of Wingbeat at the Outset and Termination of Flight

Unlike the stroboscopic method, the oscilloscope is able to track the rapid alterations in the frequency of wingbeat that occur at the outset and termination of flight. The behavior of wingbeat frequency under these conditions is recorded in Fig. 3. Two brief flights are plotted from beginning to end. In making the graphs, the total wingbeats were counted over 0.2-sec. intervals in the upper record, and over 0.5-sec. intervals in the lower one. These counts were then converted into beats per second in each case and plotted. Records of this type reveal that the early beats of the wings are accomplished at the high frequency characteristic of the entire flight.

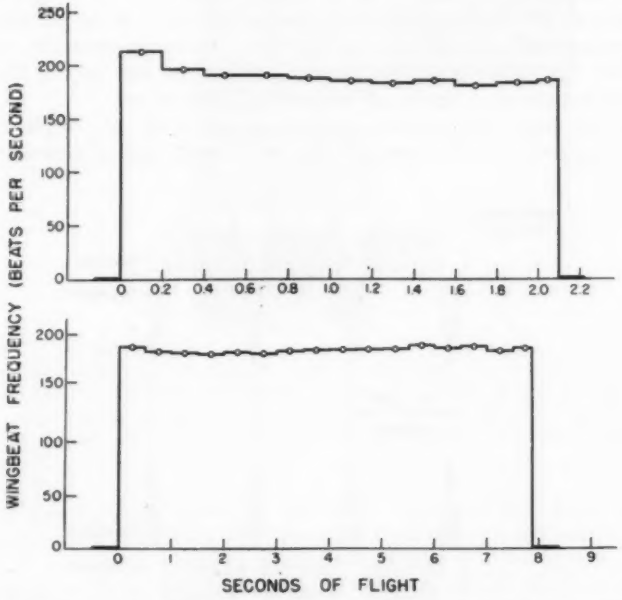

Figure 3. The frequency of wingbeat during a brief flight by each of two specimens. The frequency of wingbeat as determined from the sound records is seen to be approximately the same from the beginning to the end of each flight.

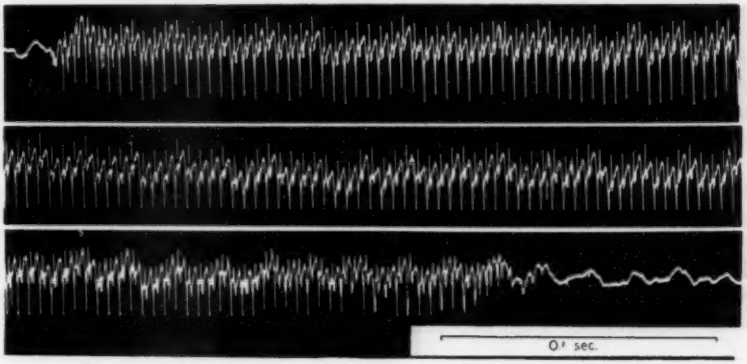

FIGURE 4. The beginning, middle, and end of a sound record of a specimen with clipped wings. The relative constancy of frequency of wingbeat, illustrated in Figure 3, is emphasized here. Irregularities at the end of the record are attributable to touching the insect with a steel rod for the purpose of terminating flight. The constant amplitude of the spikes in the middle record is an artifact due to the beam moving off the edge of the oscilloscope.

Figure 4 illustrates the sounds produced by an animal whose wings had been clipped. In this case 101 beats occur in the first 0.5 second as compared with 102 beats in the final 0.5 second. Again, the frequency of wingbeat is approximately constant from the beginning to the end of the brief flight.

DISCUSSION

Though the source of the flight sounds has long been a matter of controversy, there can be little doubt that in *Drosophila funebris* the bulk of the sound energy is the direct result of the movements of the wings. One cycle of wing motion corresponds to one cycle of flight sound. Consequently, the flight sound presents a waveform that reflects the underlying complexity of the wingbeat.

The waveforms pictured in Figure 1 are those of complex sounds. A "fundamental frequency" corresponding to the frequency of wingbeat is clear, but harmonics over a wide range are prominent and their relative strengths vary with the position of the microphone. These sounds obviously depart radically from the sinusoidal vibrations produced by tuning forks and oscillators. According to Miller and Taylor (1948), sounds very similar to these can, however, be perceived by some persons as having a pitch. The judgment is not easy to make and the accuracy with which the pure tone is matched to the complex sound is poor at best. In these facts may lie some of the subjective difficulty experienced in matching the buzz of an insect to a pure tone. Furthermore, when subjects are required to match a complex sound and a tone, a common error is to select a tone of about twice the "fundamental frequency" of the complex sound (Miller, 1950). This fact, along with the "soprano-tenor" error cited earlier, may help account for the tendency to report, from subjective flight-sound data, wing beat frequencies approximating twice the actual number (see Sotavalta, 1947, pp. 16–21). Finally, the demonstrated complexity

of the insect sound should make clear the difficulties inherent in attempts to duplicate insect sounds by generating pure tones with an oscillator or tuning fork (Roth, 1948).

Analysis of the waveform, as in Figure 2, suggests that there is remarkable aerodynamic efficiency in the total cycle of wingbeat. The insect is apparently able to extract useful flight energy, not only from the downbeat of the wings, but also from the upbeat. Approximately 85 per cent of the cycle of wing motion makes positive contribution to flight; less than one-fifth of the wingbeat cycle seems to make negative contribution to the effective, polarized flow of air produced by the beating wings.

In addition to these points of interest to the aerodynamics of insect flight, the sound picture also provides useful information concerning the underlying neuromuscular mechanism. Figures 3 and 4 illustrate that during brief flights the initial and final beats of the wings are performed at the characteristic high frequency. Evidently, the mechanism that regulates the tempo of wingbeat must be fully functional at the very outset of flight.

This observation is pertinent to a theory recently proposed by Pringle (1949) to account for the unusual physiological properties of the flight muscles of flies. According to this theory,the principal features of which have been confirmed by Roeder (1950), the movement of the wings in dipterous insects depends on a remarkable myogenic mechanism. Under the general excitatory influence of the nervous system, the muscles serving the downbeat contract when stretched by the antagonistic muscles serving the upbeat. A continuation of this process leads to movement of the wings at a tempo determined largely by the loading of the flight muscles and thus, indirectly, of the wings.

Since the wing loading, under otherwise constant conditions, is fixed largely by anatomical factors, the theory would predict that the initial wingbeat be executed at the ultimate frequency. In this respect Pringle's new theory finds confirmation in the results of the present investigation.

Summary

1. Oscillographic records are presented of the sounds produced by *Drosophila funebris* during "fixed" flight. Stroboscopic determinations of wingbeat frequency indicate that one cycle of wing motion corresponds to one cycle of flight sound.

2. The sounds generated during flight are complex and radically different from the simple harmonic motion of a tuning fork. Certain consequences of this fact are discussed.

3. Analysis of these sounds suggests a remarkable aerodynamic efficiency for the wingbeat cycle. The flying insect produces a polarized flow of air from front to rear during approximately 85 per cent of the wingbeat cycle.

4. During brief periods of flight the first few and the last few beats of the wings are accomplished at the high frequency characteristic of the entire flight. This fact is considered in relation to Pringle's new observations concerning the neuromuscular system of dipterous insects.

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PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

JUNE 21–23, 1950

The mechanism of accumulation. W. J. V. OSTERHOUT.

When a compound enters a living cell, until its inside activity becomes greater than its outside activity, work must be performed to bring this about. Such a compound may be said to accumulate. (When a single ionic species reaches a higher activity inside than outside without performance of work, as in the Donnan equilibrium, this is not accumulation as here defined.)

Experiments indicate that free ions cannot pass through the nonaqueous layer which covers the protoplasm except very slowly, but by combining at its outer surface with organic carrier molecules they can move inward much more rapidly and reach the aqueous protoplasm in contact with the inner surface of the nonaqueous layer.

Certain assumptions are made as follows. On reaching the inner surface of the nonaqueous layer, metabolic processes alter or decompose the carrier molecules so that the ions are set free. The carrier molecules which have not combined suffer the same fate so that at the inner surface of the nonaqueous layer there are practically no carrier molecules to transport ions outward. Thus the free ions are trapped in the protoplasm since they cannot pass out except very slowly.

The carrier molecules are formed in the nonaqueous layer where they concentrate at the outer surface, either because they are formed there (as is the case with cellulose and a variety of organic molecules in plant cells) or because they collect there . . . since they lower surface tension. In the latter case the surface tension is altered when the carrier molecules combine with the ions which facilitates their inward passage. In any case, they will move inward after combining since there will be an inwardly directed concentration gradient.

If we adopt this point of view we can suggest answers to some important questions such as the following:

1. Why accumulation is confined to electrolytes. This is evident since only ions will be trapped.

2. Why ions appear to penetrate against a gradient. Actually there is no such penetration since the ions enter in combination with carrier molecules. The energy needed to raise the activity of entering compounds is furnished by the reactions involved in the process of accumulation.

3. Why, in absence of injury, ions do not come out when the cell is placed in distilled water. Presumably the outgoing free ions will combine at the outer surface with carrier molecules and then move inward in the same way as ions coming from without.

4. Why the relative rate of penetration falls off as the external concentration increases. Since the concentration of carrier molecules does not increase, the rate of entrance approaches a limit.

5. Why it is not necessary for anions and cations to enter in equal numbers. When, for example, K^+ unites with a carrier molecule HR we assume that H^+ is exchanged for K^+ so that electrical neutrality is preserved. When Cl^- unites with the carrier molecule ROH we assume that OH^- is given off and RCl is formed. These reactions may take place independently.

6. Why accumulation is promoted by constructive metabolism which is needed to build up the organic carrier molecules and by destructive metabolism which brings about their alteration or decomposition.

7. Why measuring the mobilities of ions in the outer protoplasmic surface does not enable us to predict the relative rate of entrance of ions. This is to be expected if ions enter by combining with molecules at the surface. Only if K^+ is able to combine preferentially will it accumulate preferentially.

8. Why ions may come out in anoxia and at low temperatures. Presumably these conditions depress the formation of carrier molecules and their decomposition in the protoplasm more than the outgo of ions so that relatively more come out.

The role of growth and metabolism in ion accumulation of plant cells. F. C. STEWARD AND F. K. MILLAR.

The nature of the relationship between the vital properties of plant cells and their ability to accumulate salts in their vacuoles was reviewed. Earlier work with storage organs has led to the view that the ability to accumulate salts is characteristic of cells which can divide, synthesize protein and have a high rate of aerobic respiration (*Tr. Faraday Soc.*, 1937, **33**: 1006 and *Ann. Rev. Biochem.*, 1935, **4**: 519).

To elucidate the relationships between metabolic properties and salt intake, new techniques were described. By the use of plant tissue cultures, it is now possible to investigate rapidly proliferating cells in contrast with cells which, though they metabolize actively, do not grow.

The system devised (*Nature*, 1949, **163**: 920 and *Science*, 1948, **108**: 655) consists of minute cylinders of the secondary phloem of the carrot root. These discs cannot grow though supplied with a seemingly complete organic and inorganic medium. However, when supplied with a factor contained in coconut milk the tissue increases rapidly some 60–80 times in about 20 days.

Apparatus for the controlled growth of these tissue cultures and the smooth growth curve were illustrated. By treating both the proliferating and non-proliferating tissue with radioactive isotopes (Cs^{137}) ion uptake has been investigated with time.

The outstanding difference between dividing and non-dividing tissue became apparent when it was realized that (1) growing tissue took up Cs^{137} *pari passu* with growth; (2) tissue which was not *externally* growing took up Cs^{137} in a similar manner with time. These two cases of uptake, however, seemed to be different.

In the growing culture, absorption of Cs^{137} was uninfluenced by addition of a large excess of carrier Cs. Thus Cs absorbed was directly proportional to the first power of the external concentration.

In the non-growing culture, however, the Cs^{137}detected in the tissue was suppressed by addition of carrier Cs since the "absorption ratio" decreases with greater concentrations in the external solutions.

The interpretation given these facts was that there are two distinct ways in which plant cells accumulate salts. Actively metabolizing, extending cells which do not divide (cultures in absence of coconut milk) absorb by the familiar mechanism of salt accumulation in the vacuole.

Rapidly dividing cells in presence of coconut milk, however, appear to bind their Cs^{137} proportionally to the external concentration and this binding is presumably cytoplasmic.

The significance of these observations for the further investigation of the problem was indicated.

Stress and the human adrenal cortex, especially in relation to potassium metabolism. HUDSON HOAGLAND. No abstract submitted.

Potassium movement in relation to drug and ion action in nerve. ABRAHAM M. SHANES.[1]

Earlier studies (*J. Gen. Physiol.*, 1949, **33**: 75) on the behavior of the resting potential during anoxia and stimulation as modified by metabolic inhibitors, substrates, ions and stabilizing and unstabilizing drugs, have led to the conclusion that fluctuations in the polarization level of both vertebrate and invertebrate nerve are due primarily to alterations in the potassium concentration at the surface of the fibers.

Potassium movement under the same experimental conditions has now been studied directly by flame spectrophotometric analyses of small volumes of solution brought successively in con-

[1] Supported in part by research grants from the National Institutes of Health, U. S. Public Health Service, and from the American Philosophical Society.

tact with the nerves. The results confirm the predictions based on the indirect observations and thereby establish the causal relation between potassium and bioelectrical changes.

Thus, in crab (*Libinia emarginata*) and frog (*Rana pipiens*) fibers, anoxia causes a potassium release which is retarded by glucose; 1 mM iodoacetate has been found to counteract the glucose effect. Stimulation causes the well-known release of potassium. In addition, as anticipated from the large, long positive after-potential in crab nerve and from the corresponding post-anoxic over-shooting of the resting potential in both crab and frog nerve, potassium is taken up from the medium following stimulation and anoxia; the removal of the vertebrate sheath is extremely important in these experiments.

Additional details are now available for frog tissue: Cocaine (0.1%) retards the escape of potassium during anoxia, while low veratrine concentrations (0.2 to 0.5 mg.%) increase it three- to four-fold. Higher veratrine concentrations (1 to 2 mg.%), which cause depolarization directly, cause an escape of potassium which, like the electrical effect, can be prevented with cocaine.

A forty-fold decrease in the sodium content of the medium reduces the potassium escape during anoxia 30% and seriously impairs post-anoxic recovery. In Ringer's containing only 2 mM sodium—with choline or sucrose to make up the osmotic deficit—potassium release during oxygen lack is unaccompanied by an uptake of sodium, although in normal Ringer's roughly equivalent amounts of sodium enter.

The identity of the concentrations of the experimental agents which alter potassium lability and excitability, conduction, and the resting potential, suggests a common rôle for potassium movement in relation to these phenomena. In any event it appears likely that the establishment of additional details concerning the potassium shifts under a variety of experimental conditions will serve to clarify the basic mechanisms concerned with nerve functioning as well as with the mode of action of drugs and ions.

Ions and muscular contraction. A. SZENT-GYORGYI. No abstract submitted.

Potassium and sodium exchanges in human red cells. ERIC PONDER. No abstract submitted.

The disturbance by X-rays of selective potassium accumulation in human erythrocytes. C. W. SHEPPARD.

The maintenance of osmotic stability and the specific potassium accumulation of cells represents a fertile field for radiobiological research, the mammalian erythrocyte being a particularly advantageous material. When freshly drawn heparinized human whole blood is exposed to X-rays (54,000 r maximum) and then equilibrated under a controlled atmosphere at 24° C. or 38° C., (*Biol. Bull.*, 1948, **95**: 283), potassium is progressively lost from the cells and quantitatively replaced by sodium with little if any osmotic disturbance. The mean rate of loss at 20,000 r and 24° C. is about 1% of the initial cell potassium per hour and doubles approximately for every 20,000 r increase. The effect is accentuated by storage at low temperature (5° C.) following irradiation. By labeling the plasma potassium with K^{42} it is shown that although radiation slightly depresses the rate of entrance of potassium into the cells the reverse rate from cells to plasma is greatly accelerated.

Studies of normal cells with Na^{24} have shown that about half of the low intracellular sodium exchanges rapidly with that of the plasma sodium, the remainder exchanging very slowly. If cells are allowed to replace their potassium with sodium following a period of post-irradiation equilibration the rapidly exchanging sodium fraction increases in size, suggesting that this fraction is ionic sodium.

The interpretation of isotope experiments in systems of compartments is aided by the use of an electrical analogy in which the amount of material in a compartment is replaced by the capacitance of a condenser, the specific activity by the electrical charge and the exchange rate by the conductance of a resistor. Investigation of the electrical properties of an analogue to a system of four peripheral compartments surrounding a central one, shows that often the peripheral compartments may be replaced by a single equivalent substitute.

Studies on the permeability of human erythrocytes to potassium. I. M. TAYLOR AND J. M. WELLER.

In vitro studies have shown (*J. Gen. Physiol.*, 1950, **33**: 691) that the potassium ions of human red blood cells exchange with the plasma potassium ions at a constant rate, amounting to 1.5 millimoles per hour per liter of cells at 37° C. The temperature coefficient (Q_{10}) for the exchange rate is 2.2.

Observations have been made on the nature of the leakage of potassium from human red cells in the presence of fluoride and in the absence of glucose.

Studies have been made of the effect of cholinesterase and choline acetylase inhibitors. Both cause a breakdown of the potassium concentration gradient of the human red cell. Cholinesterase inhibitors decrease the rate of uptake of potassium from the plasma. Choline acetylase inhibitors increase the rate at which potassium leaves the cells.

Some ionic exchanges and ionic equilibria of the erythrocyte. M. H. JACOBS.

The erythrocyte is a particularly favorable cell for the study of this problem (a) because it shows two distinct types of permeability to ions, (b) because its ionic permeability can be experimentally modified in several ways, and (c) because its ionic exchanges are frequently accompanied by characteristic osmotic volume changes that can be followed quantitatively and recorded in detail by photographic methods.

The permeability of the erythrocyte to cations is similar to that of many other known cells in being low in degree and in involving an expenditure of energy by the cell to bring about ionic distributions that would not otherwise occur. Permeability to anions, on the other hand, is of a degree so high as to be unique among cells, is purely passive in character, and always proceeds in the direction of a predictable state of equilibrium.

The exchanges of many anions can be greatly accelerated by the proper use of the CO_2-bicarbonate-carbonic anhydrase system, and enormously and specifically retarded by low concentrations of tannic acid. Permeability to cations can be increased to an extent that can be regulated over wide limits, and passive diffusion can be made to take the place of active transport, by appropriate treatment of the cells with 5 or 6 per cent butyl alcohol.

Calculations have been made of the theoretical osmotic and ionic equilibria, in solutions containing any desired quantities of NaCl and sucrose, of hypothetical cells having the approximate chemical composition of the erythrocyte and each of the types of permeability so far experimentally realized. When compared with the theoretical predictions for such cells, the observed osmotic volume changes of both normal and modified erythrocytes show a very satisfactory semi-quantitative agreement, which extends even to unexpected details of the predictions.

The effect of physostigmine and acetyl choline on the permeability of erythrocytes. MARGARET E. GREIG.

Evidence for a relationship between the activity of cholinesterase and the maintenance of selective permeability of erythrocytes as well as certain other tissues is as follows:

1. Washed erythrocytes (dog, cat, human, beef, rabbit) which are actively metabolizing acetyl choline remain intact and maintain their original ion composition for a much longer period of time than do similar erythrocytes in the absence of substrate (acetyl choline).

2. When the cholinesterase activity of erythrocytes is inhibited by physostigmine (which is considered to be a specific inhibitor of this enzyme) changes in permeability occur at rates similar to those observed in cells without substrate; the greater the inhibition of cholinesterase, the greater the permeability to sodium, potassium and hemoglobin.

3. Substrates which are metabolized at different rates by cholinesterase had different abilities to maintain the cell intact, those which were metabolized most rapidly being most effective in preventing hemolysis.

4. A correlation between cholinesterase activity and permeability of the hemoencephalic barrier of the frog to acid fuchsin was apparent when it was found that the time of onset of convulsions caused by acid fuchsin was decreased from an average of 12.6 hours for dye alone to thirty-four minutes for those frogs in which physostigmine was administered together with acid fuchsin.

5. Physostigmine also caused local anesthetics, which had no surface activity but were infiltration anesthetics, to produce anesthesia when applied to the rabbit's cornea. There was found to be a parallelism between ability to produce surface anesthesia and ability to inhibit cholinesterase, thus indicating again that by inhibiting this enzyme the membrane is made more permeable.

6. Physostigmine decreased the lag exhibited by certain anesthetics. In the case of barbital the time of onset of anesthesia was reduced from twenty-seven minutes to eleven minutes in mice by the simultaneous administration of physostigmine. For chloralose the lag was reduced from twenty-one minutes to nine minutes. These results would indicate that physostigmine which inhibits cholinesterase increases the permeability of the blood brain barrier to anesthetics.

It thus appears that the integrity of the cholinesterase system may play an important role in cell behavior.

The role of potassium in the metabolism of E. coli. R. B. ROBERTS. No abstract submitted.

Electrolytes, metabolism and the muscle membrane potential. GILBERT LING AND RALPH GERARD. No abstract submitted.

Calcium and protoplasmic clotting. L. V. HEILBRUNN. No abstract submitted.

The influence of carbohydrates and insulin on the potassium content of leucocytes and muscle. J. F. MANERY, K. C. FISHER, D. R. H. GOURLEY, D. L. WILSON AND M. F. J. TAYLOR.

Rabbit leucocytes obtained and analysed by methods previously described (*J. C. C. P.*, 1949, **34**: 493) were shown to be relatively permeable to potassium. They readily lost most of their potassium in potassium-free Ringer's solutions and rapidly exchanged it for K^{42}. In the 45-minute centrifugation period required for packing, the specific activity of the cells was 32% of that of the fluid, while after aeration for 54 minutes plus 45 minutes centrifugation time this value was 104%.

The effect of glucose on the potassium content of leucocytes was studied. A suspension of cells was divided into two lots; one lot was exposed to a glucose-free Ringer's solution and one to a similar medium containing 160 mgm. % glucose. These suspensions were aerated at 37°C. for periods of time varying from 25 to 90 minutes. In each case the analysis of the packed cells showed that those in glucose-Ringer's solution had a higher potassium content than those in a glucose-free medium; the difference increased as the period of exposure was lengthened. Sodium appeared to exchange for potassium since a reciprocal relation was demonstrated and the cellular water concentration did not vary.

Paired frog muscles were allowed to respire in an atmosphere of oxygen in Warburg respirometers for 6 hours. At the end of the second hour experimental agents in Ringer's solution were tipped from the onset on to one member of the pair while an equivalent volume of Ringer's solution was tipped on to the control muscle. The final concentration of insulin was 0.01 units per ml. and of lactate 0.005 *M*. Analyses of both muscles and fluids for potassium showed conclusively that insulin caused muscles to take up potassium in the presence of lactate. Iodoacetate (0.00025 *M*) abolished this influence of insulin on the potassium uptake. In general the conditions under which potassium uptake occurred likewise stimulated oxygen consumption.

Studies on carbohydrate metabolism in mammalian muscle. CLAUDE A. VILLEE, VIVIEN WHITE AND A. BAIRD HASTINGS.[1]

Using techniques described previously (*J. Biol. Chem.*, **179**: 673) rat hemidiaphragms were incubated in duplicate Warburg vessels in a medium containing glucose, 11.1 mM/L., and

[1] This work was supported in part by a contract between Harvard University and the Atomic Energy Commission.

pyruvate, 10 mM/L. In one vessel the glucose was uniformly labeled with C^{14} and the pyruvate was unlabeled, and in the other the glucose was unlabeled and the pyruvate was labeled with C^{14} in the carbonyl carbon. After a two-hour incubation period, the muscle was removed and treated with boiling 30% KOH; the glycogen was isolated and hydrolyzed to glucose; glucose determinations by the Nelson method were made on one aliquot and the rest was precipitated as the glucosazone, purified, recrystallized, plated and counted with a proportional flow counter. Aliquots of the incubation medium were analyzed chemically for glucose, pyruvate and lactate and an aliquot was precipitated as pyruvate dinitrophenylhydrazone, which, after purification and recrystallization, was plated and counted. The respiratory CO_2 was recovered from the center well alkali, precipitated as $BaCO_3$, plated and counted.

Between 75 and 80 per cent of the glucose and pyruvate disappearing from the medium was accounted for as lactate appearing, glycogen synthesized, CO_2 produced, protein and lipid made, and as glucose converted to pyruvate. The remainder is apparently present at the end of the incubation as other intermediates. The rate of glucose utilization is the same in the presence or absence of added pyruvate; it is increased by the *in vitro* addition of insulin and by adrenalectomy and is decreased in muscle from alloxan-diabetic rats. The hexokinase reaction is thus not affected by the presence of pyruvate in the concentration used. However, the amount of glucose carbon metabolized to CO_2 is less when pyruvate is present, the difference being greatest in muscle from adrenalectomized rats. The amount of glycogen synthesized is increased when both glucose and pyruvate is present, over that made when either is present alone. Apparently the rate at which glucose-6-phosphate is converted to glycogen is high enough to handle both the glucose-6-phosphate made from glucose by hexokinase and that made from pyruvate by the reversal of glycolysis. The addition of insulin triples the percentage of glycogen made from glucose and decreases the percentage made from pyruvate to one-third or one-fourth that in the absence of insulin. The percentage of CO_2 derived from glucose is greatly decreased in the presence of pyruvate but the percentage of CO_2 derived from pyruvate is the same or slightly increased in the presence of glucose. These results are to be expected since the metabolism of glucose to CO_2 involves pyruvate as an intermediate.

The data obtained are in agreement with results obtained previously, that insulin has a primary effect in stimulating the hexokinase reaction and an additional, lesser effect on the reactions involved in the condensation of pyruvate with oxalacetate to enter the Krebs tricarboxylic acid cycle.

The nature of the electrolyte pump in the isolated surviving frog skin. ERNST G. HUF.

Experiments were undertaken to study the influence of ATP in Ringer's solution upon the mechanism of salt accumulation at the chorion side of surviving frog skin, with Ringer's solution of 0.4 its physiological concentration on both sides. From the results obtained, it seems that the salt pump in frog skin does not make use of the energy liberated during dephosphorylation of added ATP, although it is known from other experiments that active salt uptake by the skin is closely tied up with certain metabolic reactions. It was noticed, however, that the net accumulation of NaCl at the chorion side of the skin was diminished by ATP-sodium as compared to respective controls. Inhibition of net salt accumulation could be seen also when Ringer's solution was supplemented by either sodium-glycerophosphate or sodium-phosphate or additional NaCl. The Na^+ levels, which the skin had established at the chorion side after several hours, were nearly the same for experiments with supplemented Ringer's and for their respective controls, even when the experiments started off with a 40% higher Na concentration at the chorion side as compared to the controls. The Cl^- levels at the chorion side were the same or lower than the Na^+ levels in the experiments with NaCl and phosphates as supplements, respectively. In the latter case, phosphate replaced chloride stoechiometrically. Water moved across the skin in the direction of active salt uptake. The fluid shift was greater in the experiments with supplemented Ringer's than in the controls, except in the experiments with ATP, where the opposite relationship was found. The results seem to indicate that the skin at its inside (chorion layer) tries, first of all, to establish a constant Na^+ level. The Cl^- level may vary, depending on the presence of other anions, such as phosphate. This suggests that the

salt pump in isolated frog skin is a cation (Na^+) rather than an anion (Cl^-) pump, whose activity is regulated by the Na^+ concentration at the chorion layer of the skin.

Probable structural changes in neural excitation and the role of certain ions. JULIAN A. TOBIAS. No abstract submitted.

Experiments supporting the phase-boundary theory of bio-electricity. T. CUNLIFFE BARNES AND R. BEUTNER.

The recent important researches of Lorente de Nó (*J. C. C. P.*, 1949, **33**) confirmed our observation that tetracovalent onium compounds have extraordinary electrogenic action (*Anat. Rec.*, 1947, **99**: 618). Nerves of lobster or frog are placed in isotonic glucose. The electrolyte-free nerve is suspended between two watch glasses of glucose connected to a Speedomax Recording Potentiometer. Typical spikes are produced by adding an electrolyte first to one then to the other watch glass. The salt-free nerve is sensitized almost equally to common electrolytes such as NaCl, KCl, $CaCl_2$, $MgCl_2$, tetraethylammonium bromide and acetylcholine chloride. Considerable variation in the spike height was found but tetraethyl onium usually gave larger spikes than acetylcholine. A thread soaked in guaiacol gave smaller spikes with all electrolytes than did nerve. Frog sciatic was treated with an adrenergic oil (triacetin) which enhanced the potential produced by benzedrine.

Conduction in nerve is imitated in a glass tube containing an oil-saline interface. Recording from spaced outlets along the "nerve" tube showed that decrement of the negative wave was pronounced. The velocity is too great for ordinary recording instruments to measure. The wave is not produced by spreading of acetylcholine along the surface.

Sensitization can be demonstrated by treating the oil surface with a positive substance. In rabbit gut, the pilocarpine contraction is potentiated one-third by 3 mg. % sodium benzoate. In the oil-cell, acetylcholine has very little effect after atropine but duponal is able to partially restore the acetylcholine effect after atropine.

Complex formation in protein solutions obtained by mild extraction of skeletal muscle. WILLIAM R. AMBERSON, R. DALE SMITH, SYLVIA HIMMELFARB, CAROLYN STOUT AND HANS HOCH.

By the method of "mild extraction" proteins are extracted from whole muscles of exsanguinated rabbits (*J. Biol. Chem.*, 1949, **181**: 405). In recent studies the extracting fluid has been 10% potassium pyrophosphate solution, initially at or near pH = 10.0, but drifting to lower pH values, between 8.3 and 9.6, as extraction proceeded. This solution extracts a group of proteins, including both myogen and myosin. By the usual tests neither actin nor actomyosin has been detected in the extracts. Extraction continues for many weeks, with new solution applied periodically. At the end of three months total protein extracted amounts to approximately nine per cent of the wet weight of the muscle. The muscle imbibes fluid during extraction, but, in spite of the loss of sixty per cent of its original protein, its basic form and structure are maintained. The extracts of white muscle are clear and nearly colorless; in red muscle extracts myoglobin appears.

Electrophoretic analyses are made after dialysis against potassium phosphate buffers, usually 0.1 or 0.2 *M*, at pH = 7.6. When total protein is low the electrophoretic patterns disclose four components, A, B, C (myosin) and D, in order of increasing mobilities. At higher protein concentrations a fifth component C′ appears with mobility intermediate between C and D. When it is present the patterns are asymmetrical, with C′ much larger on the descending than on the ascending limb. When total protein concentration rises above 3 grams per cent, C′ may include sixty per cent or more of the area of the pattern. At the same time the percentage of myosin falls and the form of the D elevation is modified until it appears to fuse with the base of C′. Such extracts are viscous and show a strong positive flow birefringence.

Such observations lead to the conclusion that C′ is a protein complex, probably of high molecular weight, formed by the union of C (myosin) and D. Present evidence suggests that it is not actomyosin since (1) its viscosity is higher than that of actomyosin, (2) ATP does not

reduce the viscosity as it does that of actomyosin, and (3) it dissociates over a range of salt and protein concentrations where actin and myosin combine, or precipitate, as actomyosin. The separated D fractions give a positive Biuret test and show an absorption maximum at 260 mμ, or slightly below, a value characteristic for nucleic acid. The whole extracts show an absorption maximum at 279 mμ. It is suggested that C′ may tentatively be called "nucleomyosin."

Histological studies show that pyrophosphate extraction quickly removes the normal positive birefringence of the muscle fibers, without destroying such fine details of structure as Z lines and myofibrils. If protein diffusion space is restricted during extraction by placing the muscles in cellophane bags, birefringence persists for a longer time.

The observations are consistent with the view that normal birefringence is largely caused by the presence in the fibers of nucleomyosin, rather than by actomyosin. The hypothesis is advanced that myosin may shift from one fibrous protein system to the other, during contraction and relaxation. Extreme changes in muscle viscosity may thus be avoided. Nucleomyosin may be tentatively considered as a native protein of the living muscle cell.

Some actions of ryanodine on muscle. ROBERT RAMSEY, MARY LOUISE FLINKER AND LESLIE EDWARDS. No abstract submitted.

Experiments on spiracles. JOHN B. BUCK AND MARGARET L. KEISTER.

In mature Phormia larvae 11% of the oxygen normally taken up from air crosses the cuticle, the rest entering via the one pair each of anterior and posterior spiracles. Uptake is identical in normal larvae in air or oxygen and in larvae with anterior spiracles ligated. With posterior spiracles alone ligated, uptake is 55% of normal in air, but normal in oxygen. Further evidence that the capacity of the anterior spiracles is much lower than that of the posterior is (1) larvae with only the anterior spiracles ligated darken evenly and rapidly at pupation whereas those with only the posteriors ligated darken slowly and unevenly from the front; (2) larvae in osmic vapor blacken first posteriorly.

In larvae DDT-poisoned before ligating the anterior spiracles, the posterior spiracles pass twice the oxygen used normally, but not enough for the stimulated metabolism unless the pO_2 is raised.

The posterior spiracles are not "without closing apparatus" (Imms). Each is covered by a delicate membrane having arborizations of flat transparent fibrils and three slits overlying the traditional "spiracular openings." The slits open reversibly when perfused with glycerin.

The total area of the three slits, fully open, is only 1/60 the cross-sectional area of the tracheae leaving the posterior spiracle. If gas transport is by diffusion alone (Krogh) the disproportionate smallness of the spiracular openings makes better physical sense if the volume passed is, as in leaf stomata (Brown and Escombe), proportional to a linear dimension of the apertures, rather than to their area. Supporting this hypothesis, (1) the total slit perimeter is 2/3 the total posterior tracheal perimeter; (2) the ratio of the tracheal perimeter at the anterior spiracle to that at the posterior equals the ratio of the maximum possible oxygen uptakes from air through the respective spiracles.

A new technique for the study of biological structures in the electron microscope. THOMAS F. ANDERSON.[1]

In the conventional air drying of biological specimens for the electron microscope the surface tension of the evaporating liquid flattens all but the most rugged specimens. Such artifacts are minimized in the following method of drying in which ideally *no* phase boundary moves through the specimen. After being fixed, the specimen in water on a formvar film is dehydrated by passing it through a series of water-alcohol mixtures and eventually into amyl acetate. The specimen is then placed in a pressure bomb and the amyl acetate is flushed out with liquid carbon dioxide below its critical temperature, 32°. Then the bomb, completely filled with pure liquid carbon dioxide is warmed to a temperature *above* 32° where the liquid

[1] These studies were aided by a contract between the Office of Naval Research and the University of Pennsylvania (NR 135–197).

changes imperceptably to a gas and its surface tension vanishes. The gas is then allowed to escape from the bomb and the dry specimen is ready to be placed in the electron microscope for study.

Stereoscopic electron micrographs of such delicate specimens as bacteria, hemolysed human erythrocytes, striated muscle, protein gels, and the cilia and trichocysts of *Paramecium caudatum* show structures which do not collapse when dried by this method. A number of new morphological features have appeared, each of which merits further detailed study.

The role of phosphagen in fertilization of the sea urchin egg. EDWARD L. CHAMBERS AND THOMAS MENDE.[1]

The unfertilized eggs of *Asterias forbesi* and *Strongylocentrotus drobachiensis* were homogenized in ice cold 5 per cent trichloracetic acid, the homogenate centrifuged, and the precipitate discarded. Barium and ethanol fractionations were carried out on the extract according to the method of Le Page and Umbreit. On the alcohol insoluble fraction inorganic phosphate was determined before and after hydrolysis for one minute in 2 per cent trichloracetic acid at 100°C., and arginine was determined by the method of Sakaguchi-Weber. The quantity of arginine determined agreed closely with the quantity calculated from the inorganic phosphate liberated in one minute at 100°C. in 2 per cent trichloracetic acid, indicating the presence of arginine phosphate, in the amount of 570 μg. arginine phosphate per ml. of *S. drobachiensis* eggs and 270 μg. per ml. of *A. forbesi* eggs. Creatine phosphate could not be demonstrated.

The quantity of inorganic phosphate before and after one minute hydrolysis at 100°C. in 2 per cent trichloracetic acid (1 minute labile phosphate) and after seven minutes hydrolysis at 100°C. in 1 *N* HCl (7 minutes labile phosphate), and the quantity of total phosphate was determined on the trichloracetic acid extracts of unfertilized eggs of *S. drobachiensis* and of fertilized eggs at various intervals after insemination up to the time of first cleavage. Within the first six minutes after fertilization a prominent decrease of inorganic phosphate and a marked increase of one minute labile phosphate (arginine phosphate) were demonstrated. Further alterations in the same direction with minor variations were observed up to the time of first cleavage. The seven minute labile phosphate and the total phosphate values remained constant. The incorporation of inorganic phosphate in arginine phosphate and acid stable phosphorus containing compounds accounts entirely for the decrease of inorganic phosphate following fertilization.

Effects of nitrate on muscle and nerve responses. ARTHUR J. KAHN AND ALEXANDER SANDOW.

Isometric mechanical responses of frog sartorii stimulated by slightly supermaximal massive, transverse, twitch shocks have been recorded by the piezoelectric, cathode-ray oscillographic method for the latency events and by optical myography for peak developed tension. The normal medium was a phosphate buffered Ringer's solution, and the experimental, a similarly buffered nitrate-Ringer's (all the usual chloride replaced by nitrate). Within about 10 min. after submersion of a muscle in the nitrate-Ringer's, its behavior relative to that just previously obtained in chloride-Ringer's shows the following changes: (1) latent period events are slightly hastened, (2) the depth of the latency relaxation is increased by about 30%, and (3) the developed peak tension is increased 2 to 3 fold. The altered behavior is maintained in the nitrate-Ringer's for several hours and upon replacement of the muscle in chloride-Ringer's normal responses are restored in about 10 minutes.

The potentiated tension output in the nitrate-Ringer's is not due to recruitment of fibers, since the shocks throughout were supermaximal; nor is it due to tetanization, since the single applied shock leads to only a single action potential. It is therefore concluded that the augmented tension output in the nitrate-Ringer's results from an alteration of the fundamental mechanism of contraction of each fiber. But, since the nitrate effects are so quickly produced and reversed, it is inferred that the direct action of this agent is on the excitatory membrane of the fibers and that the potentiated mechanical response results indirectly from a modification of the coupling of excitation to the contractile mechanism. The alterations in the nitrate-latency events may be evidence for the assumed changes in coupling of excitation to contraction.

[1] Under grant from the N. C. I., U. S. P. H. S.

In pursuance of the view that nitrate directly affects excitatory events, it has further been shown that it increases the excitability of muscle fibers and also of nerve fibers of the frog sciatic trunk, and it causes an increase in the spike of the action potential of both muscle (about 25%) and of at least the alpha fibers of the sciatic nerve (25 to 75%). The potentiation of the action potential, however, has not been regularly obtained and further work is needed to establish this effect of nitrate.

Photochemical inactivation of chymotrypsin. HAROLD F. BLUM, CHARLOTTE T. POPE, JUDITH P. PRICE AND RALPH WITMER.

In activation of crystalline chymotrypsin by ultraviolet radiation absorbed by this protein ($\lambda = 0.2537\,\mu$, and polychromatic $< 0.32\,\mu$); and photosensitized inactivation with rose bengal ($\lambda = 0.546\,\mu$) and with riboflavin ($\lambda = 0.365\,\mu$) was compared.

Inactivation by ultraviolet goes on at the same rate in very low partial pressure of O_2 as in air. Photosensitized inactivation, on the other hand, is almost completely abolished in the absence of O_2; so nearly so that any residue of effect may be attributed to failure to remove O_2. These findings are in agreement with earlier studies serving to distinguish between the effects of ultraviolet radiation and photosensitization by dye (photodynamic action) in living organisms.

Some effects of x-rays on the mechanisms of cell division. TITUS C. EVANS.

It has been found in the eggs of *Arbacia punctulata* and of *Ascaris equorum* that roentgen radiation will delay division in such a way as to upset the usual chronological correlation of certain observed phenomena with the cleavage process.

Prolonged exposure of Arbacia eggs to X-radiation at low intensities allows subsequent division without further delay, upon cessation of the irradiation. This indicates a recovery rate approximately equal to the rate of inhibition. However, the longer the exposure, the more pronounced the subsequent effect on the development of the embryos. This indicates a different recovery rate for the two processes.

Irradiation of Ascaris eggs at developmental temperature allows recovery (cleavage delay) during the longer exposures. Irradiation at subdevelopmental temperatures reduces the recovery rate as regards cleavage time, but does provide recovery as regards the subsequent development of the embryos. Irradiation of eggs at developmental temperatures, with subsequent storage at 5°C. for several months, allows recovery as regards developmental injury. However, it enhances the delay in first cleavage at the longer radiation exposures.

The uptake of radioactive phosphorus in Arbacia eggs increases rapidly upon fertilization. This uptake and cleavage rate are reduced by lowering the temperature. On the other hand, irradiation will delay cleavage without affecting the rate of P^{32} uptake. Likewise, there is a sudden and rapid increase in oxygen consumption of Arbacia eggs upon fertilization which is not affected by irradiation strong enough to retard cleavage.

Direct spectroscopic observations on cytochrome oxidase and its reaction with carbon monoxide. ERIC G. BALL AND OCTAVIA COOPER.

The turbid heart muscle preparation previously described (*J. Biol. Chem.*, 1949, **180**: 113) is treated with 2 per cent solutions of desoxycholate in glycylglycine buffer pH 7.4 to yield a relatively clear preparation. This preparation is reduced by the addition of dithionite, succinate, ascorbate or ferrocyanide in an evacuated Thunberg tube fused to an optical cell. The spectrum of the reduced cytochromes is measured and carbon monoxide then admitted to the cell and the measurements repeated.

Complete and rapid reduction of all the cytochromes is obtained only with dithionite; succinate reduces cytochromes a and c completely, b partially; ascorbate reduces cytochromes a and c completely, but not b; ferrocyanide reduces cytochrome c approximately 15 per cent and cytochrome a 50 per cent. In the case of succinate, ascorbate and ferrocyanide, the reduction rate is slow unless extra cytochrome c is added. Addition of CO to preparations reduced with dithionite, succinate or ascorbate causes the band centered at λ 605 mμ to diminish and produces a new band located at λ 590 mμ; a secondary band at λ 540 mμ also appears. These reductants

also produce a band located at λ 443–445 mμ, which is most clearly defined with ascorbate. This band disappears on the admission of CO. This band does not appear in preparations reduced with ferrocyanide, nor does the portion of the band at λ 605 mμ produced by ferrocyanide shift with CO.

As a working hypothesis, it is proposed that the band at λ 605 mμ is composed of two substances, both of which function as cytochrome oxidase. One has a higher oxidation reduction potential than the other, and does not show any reaction with CO spectroscopically. The other of lower potential, not reduced by ferrocyanide, possesses a characteristic band at λ 443–445 mμ in addition to its absorption at λ 605 mμ, and both of these bands shift in the presence of CO.

Oxidase activity—light-absorption relationships in cytochrome system of heart muscle preparations. BRITTON CHANCE.

The sequence of chemical reactions between oxygen and succinate that occur in heart muscle preparations has been recently summarized by Slater (*Biochem. J.*, 1950, **46**: 484) and closely follows the mechanism of Keilin and Hartree (*Proc. Roy. Soc. B.*, 1939, **127**: 167). This sequence of cytochrome action has been based largely upon the effect of inhibitors (especially hydrocyanic acid and carbon monoxide) upon the absorption spectrum and upon the enzymatic activity of the heart muscle preparations. However, in no case heretofore has there been a direct correlation between the amount of the cytochrome component acting and the oxidase activity. By means of rapid methods for measuring oxidase activity and sensitive spectrophotometric methods for detecting the cytochrome components, studies have been made of the effect of a) oxygen concentration upon oxidase activity and upon the state of oxidation of the cytochrome components, and b) the effect of cyanide upon the oxidase activity and upon the amount of cytochrome component acting.

The platinum microelectrode (*Rev. Sci. Instruments*, 1942, **13**: 524) has been used to measure the rate of oxygen consumption, and spectrophotometry has been used at 250 mμ to measure the succinate $\rightleftharpoons$ fumarate rate and at 445 mμ to measure the optical density of the heart muscle preparation corresponding to the oxidation and reduction of Keilin and Hartree's a_3 component. The correlation of the decrease of oxygen and fumarate rates with the decrease in the concentration of oxidized a_3 component as the oxygen concentration falls to zero suggests that the concentration of the oxidized a_3 component determines the activity of the preparation. Similar conclusions are reached when a portion of the oxidized a_3 component is bound by cyanide. Preliminary numerical values are: oxygen affinity $\leqslant 1 \times 10^{-6}$ M, cyanide dissociation constant $\sim 1 \times 10^{-5}$ M, based upon both spectrophotometric and activity data.

Oxidation and reductions catalyzed by isolated chloroplasts. ALAN H. MEHLER AND HANS GAFFRON.

Isolated chloroplasts have been shown by many investigators to carry out model reactions of two components of normal photosynthesis: reduction using electrons from water, and, simultaneously, liberation of molecular oxygen. In spite of certain differences between the abilities of intact cells and isolated chloroplasts to reduce various compounds, the present studies on the mechanisms used by isolated chloroplasts are considered an approach to the understanding of the light reaction of photosynthesis.

The possibility that hydrogen peroxide might be an intermediate in the evolution of oxygen from water was tested by carrying out Hill reactions with spinach chloroplasts in the presence of catalase and ethanol, which use hydrogen peroxide very efficiently at low concentrations to form acetaldehyde. No hydrogen peroxide was detected during the course of Hill reactions. However, in the presence of air and the absence of Hill reagents, acetaldehyde was found, showing hydrogen peroxide formation by illuminated chloroplasts. It is concluded that hydrogen peroxide is not an intermediate in the liberation of oxygen, but can be formed by reduction of molecular oxygen serving as a Hill reagent.

The rate of oxygen consumption by chloroplasts with catalase and ethanol is significantly slower than oxygen evolution with quinone at equal illumination. When quinone, catalase and

ethanol are added to chloroplasts and illuminated, oxygen is first evolved at the rate characteristic of quinone, then, after about 90% of the theoretical evolution, oxygen consumption begins, and maintains a rate two to three times that of the control without quinone. The relations obtained with these systems have allowed this tentative hypothesis to be advanced: that a first reaction of electron transfer can be inhibited by a naturally occurring substance and the inhibition reversed by quinone, and that narcotics inhibit a second enzymatic electron transfer.

The influence of glycolytic factors on the potassium and sodium content of the yeast cell. GEORGE T. SCOTT AND MARC JACOBSON.[1]

The carbohydrate metabolism of the cell has been shown by a number of investigators to be associated with the accumulation and retention of potassium.

Experiments were carried out on 50 ml. samples of 2% suspensions of Fleishmann's baker's yeast in sodium enriched Williams solution placed in Goetz centrifuge tubes and maintained under anaerobic conditions. Total cell volumes were determined after centrifuging. The cells were ashed and analyzed by flame-photometry.

Time-course curves were obtained relating the loss of potassium from the cells at varying concentrations of the glycolytic inhibitors iodoacetic acid and sodium fluoride. The loss of this element in the presence of the inhibitors does not parallel the corresponding inhibition of fermentation. The data demonstrates a high sodium permeability.

In the absence of substrate in the medium approximately 30% of the maximal potassium content is lost. The remaining 70% is retained at a constant level for at least 180 hours. In the meantime the hydrolyzable polysaccharide content of the cell is declining. It is assumed the potassium retention is maintained at the expense of glycolysis.

Pyruvate given prior to sodium fluoride reduces the loss of potassium from the cell. The observation suggests the formation of a protective unit by combination of the enzyme with a compound formed from added pyruvate, or pyruvate is rapidly generating a compound which results in the retention of a significant proportion of the cellular potassium.

Anomalous penetration of phosphate into the gills of Mytilus. R. R. RONKIN.

Pieces of excised ctenidium from a single demibranch of *Mytilus edulis* L. were placed in a special glass irrigation chamber (*J. C. C. P.*, 1950, **35**: 241) in a continuous flow of artificial sea water (ASW) containing radioactive phosphorus (P^{32}) as phosphate, but no additional phosphate except for that occurring as impurities in the other components of the ASW. Such solutions contained 5.3×10^{-6} mols·l^{-1} of phosphate-P^{31} and about 1×10^{-11} mols·l^{-1} of phosphate-P^{32}. Two other solutions contained the same concentration of phosphate-P^{32}, plus added "carrier" phosphate-P^{31} to bring the total phosphate concentrations to about 100×10^{-6} and 500×10^{-6} mols·l^{-1}, respectively. Penetration of phosphate into the ctenidium from these three solutions was studied as a function of time and total external phosphate concentration, by means of a Geiger-Müller counting tube placed just above the thin glass cover of the chamber. The rate of penetration of phosphate from the ASW which contained the highest phosphate concentration (500×10^{-6} mols·l^{-1}) was two to three times higher, compared with rates of penetration from the other solutions, than could be accounted for on the basis of a direct linear relationship between penetration rate and external phosphate concentration. The order in which the solutions were administered to a given gill fragment did not affect this disproportionality. When irrigant solutions were changed, the new penetration rate was observed to be in effect as soon as a reading could be made, and reversion to the previous rate became evident immediately upon switching back to the original irrigant. The apparently anomalous penetration from the ASW with the highest phosphate concentration may be associated with the presence of particulate (colloidal) $CaHPO_4$ in the ASW. The possibilities of a greater rate of penetration owing to direct entrance of the particles into the cells, or by an increase in solubility of the salt in the immediate environment of the cell surface, were discussed.

[1] Aided by a contract with the Office of Naval Research with Oberlin College.

Effect of stretch on conduction in single nerve fibers. THEODORE HOLMES BULLOCK, MELVIN J. COHEN AND DYREL FAULSTICK.[1]

Species whose nerve fibers tolerate considerable stretch offer an opportunity to study the effect of change in physical parameters of the membrane and current path upon function. Measurements of electrical dimensions have not yet been made but should be of interest in view of preliminary results.

The median giant fiber of *Lumbricus terrestris* has been so prepared that simultaneous measurements could be made of diameter and rate of conduction under various degrees of stretch. Stimulus-response time measurements have been checked by frequent use of two-channels of pickup, proximal and distal to stimulating electrodes. These fibers will permit reversible series of estimations over a two to one range of fiber diameter. In order to make this figure accurate it was necessary to measure exactly the same point on the fiber before and after stretching, but cruder measurements without this precaution also show that diameter is actually decreasing rather than stretch merely straightening out a sinuous fiber. The result in almost every case was no change in conduction rate (per meter).

Experience in recognizing the degree of stretch necessary to assure just complete straightening permitted use of data from preparations in which diameter could not be measured. Intact, unanesthetized worms from which action potentials were recorded during spontaneous movements upon simple mechanical stimulation give exactly the same result as strip preparations of the midventral body wall or preparations of the isolated cord. One can simply take the maximum rate attained under various degrees of stretch or of extension of the intact animal as the correct figure for the straightened nerve fiber.

Three specimens, one in each type of preparation, have given anomalous results (out of more than twenty-five cases), namely, clear increases in rate. Bullock (*J. Neurophysiol.*, 1945, **8**: 55) reported increase in rate in similar experiments without diameter control. Re-examination of the scatter of points on the original graphs shows that all cases except one—that having the most points and therefore chosen for publication—permit the interpretation that two slopes intersect, the one lower on the length axis corresponding to expectation from straightening out a sinuous fiber, that higher on the length axis to constant rate. In view of the overwhelming majority of cases giving constant rate, the conclusions of that date must be amended. Thus the conclusions of Jenkins and Carlson (*J. Comp. Neurol.*, 1904, **14**: 85) and Carlson (*Amer. J. Physiol.*, 1905, **13**: 351) are confirmed, although their interpretation, that this is "evidence of the fluidity of the conducting substance," seems less satisfactory today.

We have found the same relation in the median and lateral giants in *Lumbricus*, in the third order giants of *Loligo pealii*, and in the central giants of the polychaetes *Lumbrineris erecta*, *Marphysa sanguinea* and *Eudistylia polymorpha*. Tolerance of stretch varies greatly, the squid permitting very little and the median giant of the earthworm much less than the lateral, for example.

Since core dimensions and presumably conductance can decrease greatly without depressing rate, contrary to generally accepted rules, it is to be hoped that impedance measurements will be made with a view to revealing possible submicroscopic folds and the mechanism of exact compensation of conduction rate.

[1] Grants in aid from the University Research Committee, the Rockefeller Foundation and the Public Health Service are gratefully acknowledged.

ABSTRACTS OF SEMINAR PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

JULY 5, 1950

Alkaline phosphatase activity in normal and scorbutic bones. ANITA ZORZOLI.

The deficiency disease, scurvy, is characterized in part by a disturbance in the formation and calcification of the organic matrix of the bones of the body. It has been suggested that the inhibition of calcification occurs as a result of the inhibition of the enzyme, alkaline phosphatase.

In this study young adult guinea pigs were maintained on a scorbutogenic diet for 26 days and then sacrificed for study. The proximal end of one tibia was fixed, decalcified, embedded in paraffin and sectioned for study of the histochemical localization of alkaline phosphatase. The other tibia was weighed, homogenized in distilled water and allowed to autolyze at room temperature for two days. A suitable aliquot was used for the chemical estimation of phosphatase activity.

Both chemical and histochemical methods demonstrated a significant decrease in phosphatase activity of the scorbutic bones as compared with the normal controls. Expressed as micrograms of phosphorus liberated per gram of tissue per hour of incubation, the experimental animals had an average value of 4.16 and the normal controls of 8.04. The decrease in enzyme activity was widespread throughout the bone. This was especially true of the region of articular cartilage bordering on the secondary zone of ossification and in the columnar and hypertrophic cartilage regions, where the intracellular and extracellular phosphatase activity is normally very high. As indicated by the degree of blackening, the altered osteoblasts of the scorbutic animals showed little or no phosphatase activity.

One group of scorbutic animals was given a single, large dose of vitamin C and then was sacrificed at 12, 24 and 48 hours after the treatment. At 12 and 24 hours the phosphatase activity was the same as in the untreated scorbutics. At 48 hours the activity had risen to a value in the order of 30% above the normal controls.

Reduction of Janus Green by isolated enzyme systems. S. J. COOPERSTEIN AND A. LAZAROW.

Since the supravital staining of mitochondria by Janus Green is dependent upon oxygen tension and is reversibly abolished by cyanide, (Lewis and Lewis in Cowdry's "General Cytology," 1924) a study of the reactions of Janus Green with isolated enzyme systems was undertaken. Upon reduction, the blue Janus Green is converted successively to leuco-Janus Green (reversibly), to red safranine (irreversibly) and to colorless leuco-safranine (reversibly). These changes can be followed by concomitant changes in the absorption spectra.

Components of the following respiratory chain were studied for their reactions with Janus Green and its reduction products:

$$\text{Substrate} \xrightarrow{\text{Specific Dehydrogenase}} \text{Diphosphopyridinenucleotide (DPN)} \rightarrow \text{Flavoprotein} \rightarrow \text{Cytochrome } c \rightarrow \text{Cytochrome oxidase} \rightarrow \text{Oxygen}$$

Under anaerobic conditions substrate amounts of DPN-H_2 (reduced diphosphopyridinenucleotide) reduced Janus Green only in the presence of catalytic amounts of flavoprotein (Straub's). Therefore, FLAV-H_2 (reduced flavoprotein) is the component which reduces Janus Green. Likewise dehydrogenase systems capable of reducing DPN also reduce Janus Green in the presence of catalytic amounts of flavoprotein. With the lactic dehydrogenase system the reduction of Janus Green is so rapid that it may be accomplished aerobically; with the glucose dehydrogenase system however, anaerobic conditions must be used because in the presence of oxygen the leuco-Janus Green formed is immediately reoxidized to Janus Green and visible reduction is not apparent.

Using the lactic dehydrogenase system under aerobic conditions, the addition of cytochrome *c* plus a cytochrome oxidase preparation prevented the reduction of Janus Green. Evidence has been presented suggesting that one of the reduction products of Janus Green, *i.e.*, leuco-Janus Green, reduced cytochrome *c* and thereby became reoxidized to Janus Green.

These results, therefore, indicate that whereas Janus Green can be reduced by FLAV-H_2 or any dehydrogenase-DPN-flavoprotein system, this reduction can be prevented by the cytochrome *c*-cytochrome oxidase enzyme system. The addition of these latter components would tend to keep the flavoprotein in its oxidized state, and at the same time it would reoxidize the leuco-Janus Green formed.

The reduction of Janus Green by liver cell constituents and a proposed mechanism for the supravital staining of mitochondria. A. LAZAROW AND S. J. COOPERSTEIN.

Mitochondria prepared from rat liver by either the saline or hypertonic sucrose methods reduced Janus Green anaerobically in the presence of sodium lactate. The addition of DPN or nicotinamide facilitated the reduction. The supernatant following the removal of mitochondria, or that following the removal of mitochondria plus the submicroscopic particulate components, likewise reduced Janus Green; lactate, DPN, and nicotinamide facilitated the reaction.

Janus Green forms non specific insoluble complexes with amino acids and proteins (gelatin and soluble liver proteins). Mitochondria prepared in either 0.85% saline, 0.88M sucrose, or in the latter followed by washing with saline, were equally effective in adsorbing Janus Green from a 1:10,000–1:20,000 solution, leaving little unadsorbed dye in solution. On microscopic examination of these preparations the mitochondria suspended in saline were markedly swollen granules which stained faintly blue, whereas those suspended in sucrose were deeply stained blue rods. The apparent failure of mitochondria suspended in saline to stain with Janus Green is attributed to their swelling rather than to a lack of adsorption of the dye. Likewise microscopic staining with Janus Green is not positive proof that isolated granules are mitochondria.

It has long been known that mitochondria become decolorized when oxygen is removed or cyanide is added. Lewis and Lewis (1924) found that when tissue cultures were supravitally stained with Janus Green the mitochondria became decolorized upon the addition of cyanide. Removal of the cyanide, without further addition of dye, restored the stain. Since cyanide is a known inhibitor of cytochrome oxidase, it would appear that this enzyme plays a role in the supravital staining of mitochondria.

Therefore the supravital staining of mitochondria within the intact cell appears to be dependent upon: (1) the non-specific adsorption of Janus Green on protein, (2) the rapid reduction of Janus Green in the non-mitochondrial portions of the cell, and (3) the localization within the mitochondria of the cytochrome system which slows or prevents the reduction of Janus Green at this site.

JULY 11, 1950

The enzymatic conversion of ovalbumin to plakalbumin. C. A. VILLEE, K. LINDERSTRØM-LANG, M. OTTESEN, C. B. ANFINSEN AND D. STEINBERG.

Bacillus subtilis produces a proteinase which acts on ovalbumin to split off 1.2% of its nitrogen as peptides and transforms the remainder into a second, crystallizable protein, plakalbumin. By starch column chromatography and photometric ninhydrin analyses of the effluents these peptides were separated and their constituent amino acids determined. The peptides were obtained by precipitating the protein, after a given reaction period, with 10% trichloracetic acid and extracting the TCA with ether. The aqueous solutions of peptides were lyophilized and aliquots were chromatographed with and without hydrolysis. For the first five hours of reaction, the peptides on hydrolysis yielded only valine, alanine, aspartic acid and glycine. Chromatography of unhydrolyzed peptides revealed three fractions, A, B and C, no matter what solvent mixture was used. The appearance of fractions A and C in the reaction is correlated with the ammonium sulfate titration, shown by Linderstrøm-Lang and Ottesen to be a measure of

the conversion of ovalbumin to plakalbumin. In contrast, fraction B is produced by a second reaction, not correlated with this conversion.

The peptides isolated from one column were hydrolyzed and chromatographed on fresh columns. Fraction A proved to be a hexapeptide, 1 valine: 3 alanine: 1 aspartic acid: 1 glycine; fraction B, a tetrapeptide, containing the four amino acids in equimolecular amounts; and fraction C, the dipeptide alanyl alanine.

The terminal amino acid of all three peptides was shown to be alanine by the Leonis-Levy method involving the reaction with CS_2 in alkaline solution to give a dithiocarbamic acid and the subsequent ring closure in acid solution to give a thiazalone. This is extracted with ether, hydrolyzed to give the free amino acid, and the latter identified by filter paper chromatography.

Radioactive egg albumin, prepared by incubating minced hen's oviduct in buffered salt solution containing $NaHC^{14}O_3$, and precipitated by $(NH_4)_2SO_4$ was diluted with carrier and recrystallized three times. This was used as substrate in the enzyme reaction and the peptide and plakalbumin fractions were separated as before. Aspartic acid was isolated by filter paper chromatography from the hydrolysate of each and its specific activity determined. The peptide aspartic in each of four experiments had approximately twice the specific activity of the plakalbumin aspartic. This suggests that in the synthesis of ovalbumin, the constituent amino acids do not simply fall into place on a waiting model from a uniform amino acid pool, but that the aspartic of the hexapeptide, and perhaps the whole hexapeptide portion, enters the molecule differently, either from a different peptide pool or via steps with a different rate.

The denaturation of ricin as affected by pH and temperature. MILTON LEVY AND ANGELO E. BENAGLIA.

The effects of pH and temperature on the rate at which crystalline ricin becomes insoluble in a solution adjusted to its isoelectric point can be rationalized on the following postulates: (a) A number of ionic forms of the protein related through mass action equations involving hydrogen ion exists. (b) Each of these ionic forms is characterized by a specific first order rate constant with respect to the formation of an insoluble material. (c) The theory of absolute reaction rates is applicable for the description of temperature effects on the rate constants. Summations of the individual rates with respect to the amounts of each of the ionic forms as related by the dissociation constants and pH gives the total rate for a given pH and temperature. Only a few (6) of the known acid-base groups (about 50) of ricin are of significance for the rate between pH 1 and 13 and these do not appear to be the usual acid-base groups of proteins. It is postulated that prototropic hydrogen bonds are the basis of the effects of pH upon the rate of denaturation of proteins.

The rationalization allows the description of the 1200 individual observations over a pH range of 12 units and a temperature range of 43° with fifteen arbitrary parameters. This material will appear in full in the *Journal of Biological Chemistry* in the near future.

The preparation and properties of the conalbumin of egg white. ROBERT C. WARNER AND IONE WEBER.

The presence of two protein components in the albumin fraction of egg white was first noted by Osborne and Campbell (*J. Am. Chem. Soc.*, 1900, **22**: 422) on the basis of thermal coagulation points. Longsworth, Cannan and MacInnes (*J. Am. Chem. Soc.*, 1940, **62**: 2580) found that this protein undergoes a reversible change when it is exposed to pHs below 4, resulting in a loss of solubility and change in electrophoretic mobility. Conalbumin also combines reversibly with ferric iron in a stoichiometric complex containing two atoms of iron per molecule of protein (*Arch. Biochem.*, 1946, **11**: 9). In order to study these properties in detail the preparation of purified conalbumin has been investigated. We have obtained both the iron complex and the iron free protein in crystalline form by a salting-out and alcohol fractionation method. These preparations are electrophoretically homogeneous except at low ionic strength at the isoelectric point. The iron complex has a more negative mobility at all pHs than the iron free conalbumin.

JULY 18, 1950

Amino acids and peptides as adjuvants in agglutination of starfish sperm by fertilizin. CHARLES B. METZ. No abstract submitted.

Extension of the functional life span of spermatozoa by amino acids and peptides. ALBERT TYLER.

In the course of investigation of the amino acids and sugars, of which the fertilizin molecule of sea-urchins is composed, the action of these substances on the sperm was tested. It was found that the addition of various amino acids and peptides extended very greatly the functional life span of the sperm. The amino acids and peptides thus far tested include glycine, dl-alanine, dl-valine, dl-leucine, l-lysine, dl-glutamic acid, dl-phenylalanine, l-tryptophane, l-cysteine, l-cystine, glycylglycine, and glutathione. All of these proved effective. The prolongation of functional life span amounted to greater than 50 fold in certain experiments with dilute (ca. 0.4%) sperm in 0.05 *M* glycine-sea water. Concentrations of glycine above and below this value were somewhat less effective. Optimum concentrations for the other amino acids have not as yet been determined, but preliminary results indicate that sulphur-containing amino acids are most effective in low concentration.

The animals used in this work include *Strongylocentrotus purpuratus* and *Lytechinus pictus* on the west coast and *Arbacia punctulata, Echinarachnius parma* and *Chaetopterus pergamentaceus* at Woods Hole.

The presence of the amino acids has little effect on the titer of agglutination of the sperm by fertilizin. However, the spontaneous reversal of the agglutination occurs much more rapidly. Also determinations of the liberation of antifertilizin by the sperm upon aging show that this is greatly retarded by the presence of glycine. Evidently the presence of the amino acid opposes the dissolution of this surface constituent of the spermatozoon.

Tests of the possibility that the amino acids act as nutrients showed no significant production of ammonia or of the keto-acid that would result from their oxidation. The possibility of metabolic utilization of the amino acid was also examined by determination of glycine (method of Alexander *et al., J. B. C.*, 1945, **160**: 51), after various times of incubation with sperm, and no significant change was found. It appears then that the amino acids do not serve as nutrients in prolonging the life of the sperm. Also, contrary to the prevalent view, sperm do not normally die because of exhaustion of endogenous food reserves as a result of their activity. The amino acids, in extending the life span of the sperm, maintain them in a state of high motility and high respiratory activity.

Another interesting feature of the results is that fertilization with aged sperm generally results in poor, or no, membrane elevation. In prolonging the life span of sperm, the amino acids correspondingly extend the period during which a good fertilization reaction is obtained. Evidently fertilization does not simply involve a trigger mechanism in which the spermatozoon acts in all or none fashion to pull or fail to pull the trigger. The type of response given by the egg depends not only on its own condition but on that of the spermatozoon as well.

Respiration and fertilizing capacity of sea urchin sperm in presence of serum albumin and jelly coat solution. ERIK VASSEUR, ELSA WICKLUND AND JOHN RUNNSTRÖM.

Addition of glycine or other amino acids or of serum albumin (cryst.) considerably increases the percentage of fertilization in refractive eggs of *Strongylocentrous droebachiensis, Echinocardium cordatum* or *Psammechinus miliaris.* One of the effects is the swelling of the jelly coat. The agents have, however, also proved to increase the O_2-uptake of the spermatozoa and to prolong their fertilizing life. The fertilizing capacity of a suspension of spermatozoa in sea water decreased to 50 per cent in about 100 minutes. In a similar suspension of spermatozoa in 0.2 per cent serum albumin the same decline was observed only after 14 hours. After removal of the jelly coat a higher concentration of spermatozoa is necessary to bring about 100 per cent fertilization than in eggs with jelly coat. In presence of 0.2 per cent serum albumin in

the sea water, however, 100 per cent fertilization is brought about by the same sperm concentration as that used when the eggs are surrounded by a jelly coat. In confirmation of work by Gray (1928) it was found that addition of jelly coat substance to a sperm suspension causes an increase of O_2-uptake of the spermatozoa. Whereas upon addition of albumin the rate of O_2-uptake remains increased for several hours the rate declines in presence of jelly solution to the control value within about 100 minutes (*Arkiv f. Kemi*, 1949, **1**: 393). The period of fertilizing capacity of the spermatozoa is also prolonged in presence of jelly coat solution. In one experiment with *Psammechinus miliaris* spermatozoa suspended for seven hours in 0.02 per cent jelly solution gave 100 per cent fertilization, whereas the control spermatozoa in sea water gave only 5 per cent fertilization after the same time. The activating effect of the jelly substance upon the O_2-uptake of the spermatozoa can be brought about by preparations, which are hydrolysed to some extent. Conversely the sperm agglutinating property is lost even after mild hydrolysis of the jelly.

JULY 25, 1950

The effect of metabolic poisons on potassium loss from rabbit red cells. J. W. GREEN AND A. K. PARPART.

Freshly withdrawn rabbit red cells were washed in isotonic NaCl solutions which were buffered with 0.03 *M* phosphate. Washing was continued until the content of reducing substance in the cell suspensions was 15% of that in whole blood. Subsequently, this 15% decreased at the same rate in the presence or absence of inhibitor. Paired aliquots of these cell suspensions, to one of which glucose in the amount of 400 mg.% was added, were exposed to the action of 0.05 *M* sodium fluoride (NaF), 0.0005 *M* sodium azide (NaN_3), 0.0003 *M* iodoacetic acid (IAA) and 0.0001 *M* p-chlormercuricbenzoic acid (PCMB) under sterile conditions at pH 7.0 and 36.8°C. for times up to 48 hours. PCMB was very lytic and only one determination of K loss was made. The following table shows the times in hours to 40% loss of initial K value:

Poison	Cells with glucose	Cells without glucose
Controls	34	25
PCMB	0.3	0.3
NaF	13	14
IAA	16	17
NaN_3	26	19

The relatively slower loss of K in the control with glucose over that without glucose indicated that glucose added to the environment was a factor in K retention in red cells at pH 7.0. However, from the table it is clear that NaF, IAA and PCMB produced about the same rate of K loss in the presence as in the absence of glucose. This result is interpreted to mean that in these experiments the chief action of these metabolic poisons was an alteration of the cell surface rendering it more permeable to the loss of K. NaN_3 did not have this effect to the same degree.

The effect of x-rays on permeability to water and to glycols of Arbacia eggs. B. LUCKÉ, R. RICCA AND A. K. PARPART. No abstract submitted.

The permeability of erythrocytes at different temperatures. M. H. JACOBS AND H. N. GLASSMAN.

It is a common belief that a high Q_{10} temperature coefficient for the entrance of a solute into a cell indicates that a chemical mechanism of some sort is involved in the process. On the other hand, Danielli and Davson have given plausible reasons for the view that such temperature coefficients might have a purely physical origin. The present observations on the permeability of the erythrocytes of several dozen species, representing all the large classes of vertebrates, to 4 solutes (ethylene glycol, glycerol, urea and thiourea) at temperatures of 0, 10, 20, 30 and 40°C. support the physical rather than the chemical theory. In general, there is nothing

characteristic about the temperature coefficient for a given solute. Urea and glycerol, in particular, show a wide and continuous range of Q_{10} values from group to group and from species to species. These values, however, in nearly all cases are closely correlated with the difficulty of entrance of the solute into the erythrocytes in question.

In making simultaneous comparisons of many different species, solutes and temperatures, the ordinary method of tabulating the experimental data and the temperature coefficients derived from them is prohibitively cumbersome. Instead, a graphic method has been found to be very satisfactory, inasmuch as it shows at a glance both the details and the broader general relations contained in a given set of data, and at the same time it may be made accurately quantitative. The method is to draw for each species 4 lines at right angles to each other, one for every solute. On these lines the observed times of hemolysis in isosmotic solutions of the solutes, at different temperatures, are plotted logarithmically, and the points corresponding to each temperature are connected to form a quadrilateral. Under the conditions here in question, and with certain qualifications discussed elsewhere, the permeability ratio for a given 10 degree interval is approximately equal to the reciprocal of the corresponding time-of-hemolysis ratio. Because of the logarithmic method of plotting, the same distance between two points representing an interval of 10 degrees everywhere corresponds to the same Q_{10} ratio. General comparisons of the diagrams and all their details can therefore readily be made by eye. For greater accuracy an appropriate logarithmic scale permits the exact values both of times of hemolysis and of Q_{10} ratios to be read off, while a similar logarithmic scale magnified 4 times and covering a correspondingly shorter range gives directly the mean Q_{10} value for the entire 40 degree range of observations.

August 1, 1950

Orientation to heat and light during morphogenesis in the amoeboid slime molds. John Tyler Bonner. No abstract submitted.

Polarized light and the orientation of bees. Donald R. Griffin.

Independent confirmation was reported of certain key points among the recent discoveries of K. v. Frisch concerning the dances and orientation of bees (*Experientia*, 1946, **2**: 397; 1949, **5**: 142; 1950, **6**: 210; and *Naturwissenschaften*, 1948, **35**: 12; 1948, **35**: 38). These included (a) the shift from the round dance to the figure eight shaped "Schwanzeltanz" as a source of sugar solution was moved from 10 to 100 meters from the observation hive, (b) a markedly greater number of turns of the Schwanzeltanz per unit time in bees returning from 100 meters as compared with others bringing pollen from much greater distances, (c) the dependence of the direction of the straight portion of the Schwanzeltanz on the position of the sun in the sky, and (d) the effect of a polaroid sheet in shifting the direction of this dance when it is being performed on a horizontal surface with only a small area of blue sky visible to the bees.

A piece of polaroid alters the brightness and color pattern of the blue sky. The possibility was therefore considered that these effects, rather than the change in plane of polarization, might account for the turning of the dances. Such an explanation, however, seems unlikely to be correct, because of the small magnitude of the differences in brightness and color within the area of blue sky which is sufficient to orient the dances and because of the reported differential brightness and color thresholds of bees (*J. Gen. Physiol.*, 1933, **16**: 773; *Zeitschr. f. vergl. Physiol.*, 1927, **5**: 762). One is forced to conclude that bees are indeed able to detect the plane of polarization of the light from the blue sky.

On the nature of Cypridina luciferin. Aurin M. Chase.

The Japanese ostracod crustacean, *Cypridina hilgendorfii*, ejects an enzyme, luciferase, and a substrate, luciferin, into the sea water, producing a blue luminescence; probably an oxidative reaction. Luciferin also undergoes non-luminescent oxidation without luciferase (Harvey, "Living Light," Princeton University Press, 1940).

Cypridina luciferin has been partially purified (Anderson, *J. Gen. Physiol.*, 1935) and many of its chemical properties have been investigated by various workers. On the basis of these data it has been assumed to be related to the naphtho- or benzohydroquinones. The as yet unisolated

luciferin of luminous bacteria has been assumed to have a similar structure, largely on the basis of absorption spectra calculated from photochemical inactivation of bacterial luminescence (Spruit, *Enzymologia*, 1949).

Because of the need for an accurate absorption spectrum of Cypridina luciferin, measurements have now been made in the visible and ultraviolet, using the Beckmann spectrophotometer and solutions of luciferin prepared from Cypridina by Anderson's method, with further treatment designed to remove lipid impurities. The resulting spectrum is unstable and changes during exposure of the solution to air. Measured in either pH 6.8 phosphate buffer or methanol, the initial spectrum shows a sharp maximum at about 265 mμ, a shoulder at about 310 mμ and a broad absorption band in the visible, centering at about 435 mμ. On standing in phosphate buffer the 265 mμ peak decreases markedly, while simultaneously a new absorption band appears at about 365 mμ. These changes are quantitatively related to the rate of disappearance from the solution of luciferin capable of giving luminescence upon addition of luciferase. The original absorption band at 435 mμ is replaced by one at about 465 mμ, which subsequently disappears.

Chase and Gregg (*J. C. C. P.*, 1949) have recently shown that nitrogen may be present in Cypridina luciferin, contrary to an earlier statement in the literature. While, therefore, the absorption spectrum of these luciferin solutions and the changes which occur during exposure to air indicate a striking similarity to naphthohydroquinone derivatives, it is possible that Cypridina luciferin may be a nitrogen-containing compound such, for example, as the pterins or pyrimidines.

Reactions catalyzed by isolated chloroplasts. ALAN H. MEHLER.

The mechanism involved in transferring the hydrogen produced in the light reaction of photosynthesis to the carbon dioxide reducing system is completely unknown. In experiments designed to separate these reactions, McAlister and Myers and Auftemgarten found a pickup of CO_2 following cessation of illumination. After illumination in the absence of CO_2, Calvin and Benson demonstrated that algae rapidly incorporated labelled CO_2 into the same compounds found labelled after short periods of photosynthesis. The latter authors ascribe their results to the photochemical formation of a long-lived general reducing compound. The following two reasons are given, however, as evidence that no such special compound exists.

First, the data of Calvin and Benson, showing fixation of CO_2 equivalent to about 6 seconds of photosynthesis, indicate a concentration of the hypothetical reducing compound of the order of magnitude of the chlorophyll concentration in the cells. No evidence for a stored reducing power was obtained with spinach chloroplasts in experiments capable of detecting reduction in amounts 1% of the amount of chlorophyll used, measuring reduction of 2,6-dichlorophenol indophenol or cytochrome c spectrophotometrically. If the reduction step of photosynthesis used a carrier present in the amounts calculated, the chloroplasts isolated might be expected to retain enough to detect.

Second, using pre-illumination and tagging techniques similar to those of Calvin and Benson, Fager has shown that essentially all of the label fixed appears in glyceric and pyruvic acids. This indicates that the reduction of CO_2 in pre-illumination experiments is limited to one step of photosynthesis.

It is concluded that pre-illumination does not result in the production of a generallized reducing power, but in changes of concentration of photosynthetic intermediates to a pattern permitting dark fixation of CO_2 in phosphoglyceric acid.

AUGUST 8, 1950

Some spectrophotometric observations on invertebrate nerves and their extracts. A. M. SHANES AND T. J. DEKORNFELD.[1]

Suitable modifications of the Beckman spectrophotometer have permitted a study of the selective absorption of visible light by intact fin nerves of the Squid (*Loligo pealii*) and by intact leg nerves of the spider crab (*Libinia emarginata*) in the presence and absence of oxygen.

[1] Supported in part by a research grant from the National Institutes of Health, U. S. Public Health Service.

Observations have also been made on an ultraviolet absorbing component which appears in the sea water in contact with the nerves and which can be obtained in larger quantity by distilled water extraction. The absorption by these aqueous extracts was also examined in the visible range.

A number of distinct peaks become apparent between 540 and 570 mμ when the nerves or their extracts are rendered oxygen free; they are also seen in the presence of oxygen if the reducing agent sodium hydrosulfite is added. These changes are reversible.

Aqueous extracts prepared by soaking about 50 mgm. nerve tissue for 30 minutes in 15 ml. of distilled water give a single, sharp peak at 271 mμ, the density being about 0.5 in a 10 mm. cell. This peak, practically insensitive to changes in pH (3.0–10.3), was also observed in Loligo axoplasm kindly furnished by Drs. M. A. Jakus and A. J. Hodge of the Massachusetts Institute of Technology. The substance responsible for this ultraviolet absorption is dialyzable and is not precipitated by perchloric acid; the Biuret test proved negative. Different peaks were found with crab muscle extracts and crab blood.

Selective damage to fibroblasts by desoxycorticosterone in cultures of mixed tissues. IVOR CORNMAN.[1]

Fetal or newborn mouse heart in roller tube cultures gave rise to cells of the fibroblast type and sheets of cells resembling endothelium. Fibroblasts predominated, being the only cell type growing from 414 explants, while fibroblasts combined with endothelium grew from 144 explants, and endothelium alone from 12. DOC at 0.02–0.1 mg./ml. damaged both types of cells, but the fibroblasts reacted first, shrivelling to elongate filaments. Regeneration from the explants was predominantly endothelial. The treated cultures before exposure to DOC included 30 explants with fibroblasts alone, 28 with fibroblasts and endothelium, and 1 with endothelium alone. New growth following DOC treatment gave 11 explants with fibroblasts alone, 14 with endothelium and fibroblasts, and 34 with endothelium alone. It is concluded that cells of the fibroblast type are more quickly affected by DOC and recover less readily than the endothelial type. Cortisone added to DOC produced more severe damage of both cell types. The selective effects of DOC on fibroblasts *in vitro* can be compared with the specificity of DOC in producing fibrous lesions of a rheumatic type *in vivo*. Further tests must be made to determine whether the selective damage to fibroblasts in culture is peculiar to DOC or is merely a nonspecific steroid effect.

Ionic balance required by some marine microorganisms and effect of quaternary ammonium ions. WOLF VISHNIAC, ROY KISLIUK AND SEYMOUR H. HUTNER.

Cultivation of obligately marine microorganisms on synthetic media reveals requirements for the various properties of marine environment: osmotic pressure, high Na concentration, and ratio of Na to other ions.

The two marine agar-digesting cytophagas described by Stanier in 1941 were grown on a medium containing 3% NaCl, but other marine cytophagas which have been isolated since then required Na, Ca, Mg, and K in approximately the ratio in which these ions occur in the sea. Other marine bacteria require mainly the maintenance of osmotic pressure. *Pseudomonas droebachense* and several strains of unidentified luminous bacteria grow well in media containing at least 1.5% NaCl; however, the NaCl level may be lowered to 0.1% if osmotic pressure is maintained with 12% sucrose. An obligately marine pathogenic organism, the causative agent of "speck finger" (to be described elsewhere as *Micrococcus thjöttii*), probably behaves in a similar manner.

Experiments were conducted especially with a marine chlamydomonas, which, though largely indifferent to changes in osmotic pressure, showed a definite requirement for Na. The lower limit was usually 0.075–0.1% NaCl, depending on the balance of other ions. Mg permitted better growth at lower Na concentrations, K raised the Na requirement slightly, while in the presence of NH_4^+ high Na concentrations were required for growth.

[1] This work was financed by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University, Washington, D. C.

At limiting NaCl concentrations the addition of 0.05–0.1% tetraethylammonium bromide (Et_4NBr) increased growth of the chlamydomonas by a factor of 3–5; Et_4NBr also permitted growth at a slightly lower Na level (0.05% NaCl). At best there is therefore only a partial replacement of Na by Et_4N^+ for the growth of marine organisms, in contrast to the claims of de Nó concerning nerve excitation. Synaptic blocking by Et_4NBr is probably an effect independent of any relationship to Na.

The control of elongation in the hypocotyl of the kidney bean. WILLIAM P. JACOBS.

Two regions of maximal growth were found in the hypocotyl of kidney bean seedlings. The first region was dominant during the first few days of seedling growth, the second region during the later growth. By measuring the growth of isolated sections, it was shown that the second region can grow before it normally does in the plant if it is given an external supply of auxin and sucrose. By direct test using the *Avena* bioassay, it was shown that the second region contains negligible amounts of diffusible auxin, that auxin can not be transplanted acropetally through any region of the hypocotyl, but that it can be transported basipetally. Hence, auxin for the growth of the second region probably comes from above. It has long been known that cotyledons and/or the epicotyl are sources of diffusible auxin. For auxin from the cotyledons and epicotyl to reach the second region it must pass through the first region. Therefore, the transport properties of the first region were studied. It was found that no auxin was transported across the first region in the 3-day or earlier stages of seedling growth, that an increasing amount could be transported in successively older seedlings. Hence, it seems probable that the second region, needing auxin to grow, is being limited in its growth by the auxin-transport ability of the region standing between it and its auxin supply. This conclusion is confirmed by the very high correlation coefficient (0.94) between the growth of the hypocotyl and the amount of auxin which can be transported through the first region.

AUGUST 15, 1950

The sensitivity to light, reactions to shading, pigmentation, and color change of the sea urchin, Diadema antillarum Philippi. NORMAN MILLOTT.

That certain sea urchins react to changes in light intensity has long been known. The black urchin *Diadema antillarum*, common around the coast of Jamaica, responds by vigorous spine movements to both increase and decrease in intensity, but as the responses to the latter are more marked and regular, they have been studied exclusively.

The response has a latent period of 0.6–1.4 seconds and is oriented, most of the spines converging toward the object casting the shadow. A sharp shadow is not essential, diminution in intensity of illumination is sufficient to elicit the response, provided it occurs rapidly. Removal of a section of the nerve ring, although affecting the tone of the spine muscles, does not abolish the response to shading, which can be obtained in isolated pieces of test, provided that the radial nerve strand and its lateral branches remain intact. Stimulation by isolated light spots 0.5 mm. and 1.5 mm. in diameter shows that the spines are insensitive. The whole surface of the test and peristome is sensitive, but not equisensitive, the order of sensitivity being ambulacral margins → ambulacral center → interambulacrum. There is a significant relationship between sensitiveness and pigmentation, darker regions being invariably more sensitive than paler and the order of sensitiveness and degree of pigmentation correspond exactly. The receptors are located outside the test and have a wide distribution, the latter discovery excluding the possibility of structures which have previously been described as eyes in allied forms, from being the sole photoreceptors. The extensive superficial nerve layer may therefore be stimulated directly by changes in light intensity, and the response has the characteristics of a reflex with the centers in the radial nerve strands.

Diadema shows a color change which is most marked in young forms, being lighter by night, certain parts of the test becoming completely white and forming a pattern, which, during intermediate phases of the color change, appears a brilliant blue, due to dispersion effects of the receeding black pigment. As the animal becomes lighter its sensitivity to shading undergoes

a marked increase. The color change is rapid (about 1½ hours is required for the full range) and largely due to changes in large skin chromatophores, which are highly branched in the expanded phase and globular in the contracted; their behavior is independent of the central nervous system and localized expansion of the contracted chromatophores can be elicited by projecting a light spot on them. Despite the obvious correspondence between depth of color and light intensity, there is an underlying, diurnal rhythmic color change, for forms kept in total darkness become slightly darker by day.

The black pigment gives the reactions characteristic of melanin, and may be formed in the amoebocytes of the coelomic fluid which darken on exposure to air, especially after irradiation with ultraviolet and if a trace of catechol be added to the coelomic fluid. The coelomic fluid will oxidise catechol and "dopa" *in vitro*, but feebly, at pH 7.4 and 30°C.

The fact that a more rapid oxidation of "dopa" is achieved by a water extract of the amoebocytes, separated by centrifuging from the coelomic fluid, suggests that an inhibitory factor to phenolase activity exists in the coelomic fluid. The rapidity with which 0.01% methylene blue injected into the coelom is reduced, suggests that the inhibitory factor might be associated with a system of low oxidation-reduction potential, while the abolition of the inhibitory effect by methyl alcohol is somewhat suggestive of a dehydrogenase.

Photorecovery after ultraviolet radiation in amphibian larvae. HAROLD F. BLUM AND MARGIE M. MATHEWS.

Photorecovery after ultraviolet radiation was demonstrated in the larvae of *Amblystoma*. The larvae were exposed five days per week to sub-lethal doses of intense ultraviolet radiation (λ 0.23 μ to 0.313 μ). When the larvae were illuminated with "visible" radiation from fluorescent lamps after the exposure to ultraviolet, they survived longer than if kept in the dark. Thus, visible radiation enhances recovery from the effects of the ultraviolet. Larvae illuminated with visible radiation for five hours immediately after each dose of ultraviolet, survived longer than larvae illuminated with visible radiation for five hours just prior to dosage with ultraviolet. The latter survived longer than larvae kept in the dark. This shows that recovery is not yet complete 19 hours after the exposure to ultraviolet, and that illumination with visible radiation is still effective at that time. The longest wavelength causing photorecovery is about 0.5 μ. The short wavelength limit is not yet established, but wavelengths in the near ultraviolet are effective. Thus, the wavelengths for photorecovery are restricted to the same spectral region as "photoreactivation" in fungi and bacteria (Kelner; Dulbecco), and photorecovery of cleavage rate in sea urchin's eggs (Blum, Loos and Robinson). The prolonged recovery process, and the absence of effect of visible light on the normal organisms are also paralleled in the above instances. All appear to represent the same fundamental process; the repair of effects of ultraviolet radiation by a specific range of wavelengths extending from the very near ultraviolet to the blue. The present instance seems to be the first described in a vertebrate, extending the known distribution of this phenomenon in the animal kingdom.

Action of alcohol dehydrogenase on vitamin A. ALFRED F. BLISS.

Retinene is a fat-soluble yellow substance first obtained from the photolabile visual pigment, rhodopsin (visual purple). It has been identified by Morton as vitamin A aldehyde, and is reduced to vitamin A alcohol by various tissues, including the retina and intestinal mucosa. Wald has shown that coenzyme I is required for the reaction in the retina. We have shown that the reaction occurs in solutions of bleached visual purple and is reversible (*Biol. Bull.*, 1949, **97**: 221), both in isolated retinal rods and in liver extracts containing alcohol dehydrogenase. The reversibility of this step shows that the regeneration of visual purple from vitamin A can procede via vitamin A aldehyde.

In the present experiments, the equilibrium constant of the dehydrogenation of vitamin A by coenzyme I was determined with the aid of alcohol dehydrogenase obtained from horse liver by a modification of the method of Bonnichsen and Wassen (*Arch. Biochem.*, 1948), involving fractionation by ammonium sulfate between 50 and 60 per cent saturation and by alcohol. A linear relation between log k and pH was obtained, as found by Racker (*J. B. C.*, 1950) for ethyl alcohol. The dehydrogenation of vitamin A was much more extensive than that of alcohol, the respective k_{II} for each being $3.3 \cdot 10^{-9}$ and $1.15 \cdot 10^{-11}$.

The regeneration of visual purple from vitamin A aldehyde in solutions of bleached visual purple was dependent on a thermolabile factor extracted from the melanotic pigment layer of the frog eye by dilute NaCl. An association of the factor with the protein fraction was indicated by its resistance to dialysis and by precipitation with ammonium sulfate and alcohol, with recovery of about 50 and 25 per cent, respectively, of the initial activities (*Fed. Proc.*, 1950).

August 22, 1950

Population dynamics in Escherichia coli. Lillian K. Schneider.

The mutation from histidine dependence (h-) to histidine independence (h+) in the bacterium, *Escherichia coli*, has been shown to be spontaneous by application of the Luria and Delbruck variance analysis. The growth rate and final level of growth of h− on medium with optimal histidine (25γ/cc.) are the same as h+ with no histidine (H−) and with 25γ histidine/cc.(H+). Mixtures of h− and h+ grown on H+ show no shift in ratio after only twelve generations. Definite shifts occur over a greater number of generations.

Cultures of pure h− bacteria, passed through hundreds of generations by serially transferring 0.5 cc. of a fully grown culture to 50 cc. of fresh H+ every twelve hours, show an increasing number of h+ back mutants until an equilibrium ratio of $1h+/10^6h-$ is attained. This equilibrium has been maintained for more than four hundred generations in several different experiments. The forward and back mutation rates are not sufficient to explain the ratio observed. A selection pressure of 0.02 against the h− has been calculated.

Cultures started at high h+/h− ratios, by making artificial mixtures, show a long period with no shift and then a rapid drop in h+ ratio until the same equilibrium value as above is reached. This has been shown to be due to the selection of a fast growing h−. The equilibrium is reached with the appearance of a fast growing h+ from these fast h− bacteria. An unusual feature of this situation is that although a new h− is selected for, the same equilibrium value is reached. Independent measurement of the selection pressure against h+ and a more precise definition of the selection are now being studied.

Heterokaryosis between opposite mating types of Neurospora crassa. Samson R. Gross.

Heterokaryosis between like mating types in *N. crassa* is a well known phenomenon; its occurrence between opposite mating types has been subject to controversy. To demonstrate heterokaryosis between opposite mating types four laboratory stocks were used: lysineless albino *a* (7S2a), pantothenicless albino *a* (26a), pantothenicless pink *A* (5531A), and lysineless pink *A* (4545A). Two heterokaryons were prepared by mixing conidia of 26a and 4545A, and 7S2a and 5531A on minimal Fries 2% agar slants and incubated at 25° C. To prove that heterokaryosis rather than syntrophy alone had occurred, single hyphae were isolated. Fifty per cent of the isolates proved viable on minimal medium. They crossed freely with both *A* and *a* tester strains and also *selfed*. Plating macroconidia obtained from these cultures on sorbose medium (a medium causing restricted growth) supplemented by pantothenic acid or lysine, indicated the presence of both nuclear types.

The growth rate of these heterokaryons is slow and irregular. Studies of the ratios of the homokaryotic conidia in this heterokaryon indicate that the slow rate of growth may be due to an alteration of the ratios of the two nuclei so that the organism is, in effect, growing on a limited amount of growth factor. Ratios of homokaryotic conidia obtained from heterokaryons between like mating types are from two to eight pantothenicless to one lysineless. If these conidial ratios reflect the ratios of nuclei in the mycelia then there is an effective range of ratios of pantothenicless nuclei to lysineless capable of maintaining the organisms at a normal growth rate. In the heterokaryon 7S2a : 5531A the ratio of conidia is found to be 20 to 80 to 1 and for the 26a : 4545A heterokaryon 400 to 1200 pantothenicless to one lysineless.

The pantothenicless conidia, then, are normally in excess when the mating types are similar. They are more strongly in favor of the pantothenicless when the heterokaryon is between opposite mating types; and there is greater variation in the nuclear ratios depending upon whether the *a* is part of the pantothenicless genotype rather than the lysineless. We may infer from this

fact that attempts to study dominance relationships in heterokaryons should be approached with caution since nuclear ratios often may be determined by factors other than the auxotrophic mutants under investigation.

The homology patterns of induced lethal mutations in Neurospora crassa. K. C. ATWOOD.

Ultra-violet induced lethal mutations were obtained and preserved as heterokaryons by a method previously described (*Biol. Bull.*, 1949, **97**: 255). In an attempt to estimate the number of identical mutations in a series of 156 independently induced lethals, each mutant was tested for homology with every other mutant. This was done by forming a heterokaryon between two heterokaryons each carrying a lethal in its amycelial component. The conidia of this secondary heterokaryon were then streaked on sorbose medium, and the appearance of amycelial colonies in the streak where none appeared in control streaks of the primary heterokaryons was taken as a certain indication that the lethal mutations were not homologous. Since the secondary heterokaryons were not forced, the cases where amycelial colonies failed to appear can be interpreted in two ways: either one of the component primary heterokaryons excluded the other, or the lethal mutations in the primary heterokaryons were homologous. Of 1746 tests made to date within five groups of 14, 16, 29, 49, and 47 mutants, 1488 or 85.3 per cent showed the non-homologous reaction.

To the extent that homology as determined by this represents simple allelism, one would expect that two homologous mutants would show identical patterns of homology when tested against other mutants within a group. Actually, this type of internal consistency was not found. None of the mutants showed identity of reactions within the group.

Certain mutants, however, appeared to be simultaneously homologous with a large number of others which were mutually non-homologous. These can be interpreted as large chromosomal deficiencies which overlap many smaller, separate deficiencies or many loci. Assuming that there are deficiencies, the minimum number of loci which must be postulated to account for the pattern was found to be slightly greater than the number of mutants tested.

The alternate interpretation, *i.e.*, that homologous reactions are false, implies in all groups a number of loci equal to the number of mutants tested. In short, no matter how the pattern of homology is interpreted, the conclusion is forced that the number of loci in Neurospora is very large.

Comparison of the base composition of nucleic acids of nuclei and cytoplasm of different mammalian tissues. ALFRED MARSHAK.

After separation of nuclei and cytoplasm and extraction with cold TCA and with fat solvents, aliquots were digested with $HCLO_4$ and the nitrogenous base content determined by paper chromatography and spectrophotometery. Other aliquots were subjected to the Schmidt-Thannhauser procedure and the bases determined on the DNA and the PNA-nucleotides so separated. Recovery in DNA and RNA agreed with that of the unfractionated materials within 1–4% for all bases except guanine where a single 10% deviation was found. Previous experiments showed that recovery of bases from $HCLO_4$ digests of nucleic acids was quantitative (96–104%).

The base ratios of DNA from nuclei of all tissues were the same within the limits of error. As a group the nuclear PNA's (N–PNA's) had base ratios strikingly different from those of the cytoplasmic PNA's (C–PNA's). N–PNA of thymus was exceptional in having a very low cytosine content. Heart C–PNA had no detectible uracil. (The value given in the table is for some unidentified substance with the same Rf as uracil, but with a different absorption spectrum.) The structural difference between N–PNA and C–PNA may be correlated with the difference in function of the two nucleic acids previously reported. The marked differences in the base ratios of the N–PNA's of different tissues implies that cellular differentiation involves chemical change of nuclear constituents as well as cytoplasmic changes. The low adenine content of the C–PNA's suggests that the free adenine nucleotides of the cell may be liberated on the conversion of N–PNA to C–PNA, since previous experiments show that the former may be the precursor of the latter, while the reverse is not possible. The guanine-

TABLE I

Ratios of mols of purines and pyrimidines with adenine as unity

	Guanine	Cytosine	Thymine	Uracil
Nuclei				
DNA				
Liver	0.89	0.85	1.04	—
Thymus	0.89	0.79	0.99	—
Kidney	0.79	0.78	1.00	—
Heart	0.82	0.82	1.01	—
PNA				
Liver	1.00	0.39	—	0.45
Thymus	0.88	0.09	—	0.17
Kidney	0.92	0.25	—	0.59
Heart	0.92	0.19	—	0.29
Cytoplasm				
Liver	1.83	1.22	—	1.12
Thymus	1.88	1.44	—	0.89
Kidney	1.78	1.25	—	0.94
Heart	1.20	0.77	—	0.03 (?)

adenine ratios of the C–PNA's may be accounted for by the loss of one mole of adenine from the N–PNA's.

GENERAL SCIENTIFIC MEETINGS

AUGUST 29–30, 1950

Oxidative phosphorylation by a cell-free, particulate, enzyme system derived from fertilized Arbacia eggs. PHILIPP STRITTMATTER, A. K. KELTCH, C. P. WALTERS AND G. H. A. CLOWES.

During the summer of 1949 a cell-free, particulate, oxidative, phosphorylating system was obtained from unfertilized but not from fertilized Arbacia eggs. A comparison was made between the action of a series of nitro- and halophenols on the respiration and cell division of fertilized Arbacia eggs and on the respiration and phosphorylation of the cell-free, particulate system obtained from unfertilized Arbacia eggs. Since any comparison of these effects rests on the assumption that the cell-free, particulate systems derived from unfertilized and fertilized Arbacia eggs have similar phosphorylating mechanisms, it appeared desirable to make further attempts to obtain an oxidative, phosphorylating system from fertilized Arbacia eggs.

When the procedure found successful in preparing the particulate system of unfertilized Arbacia eggs (*Biol. Bull.*, 1949, **97**: 242) was attempted with fertilized eggs, it was found necessary to force the suspension through the needle fifteen to twenty times in order to rupture the egg membranes, thereby resulting in complete disintegration of the cell contents. As a result of this more violent treatment or perhaps some less obvious variation, the supernatant was totally inactive as a phosphorylating system. Little success was achieved by varying the potassium chloride and citrate content of the homogenizing solution, adding cysteine or glutathione to protect sulfhydryl groups or heparin to inhibit coagulation, or by maintaining a temperature as close to 0° C. as possible.

Finally, a more drastic procedure was employed. The fertilized eggs, after two washings with sea water and centrifuging, were chilled and shaken for less than a minute with a cold, aqueous solution containing 0.72 *M* urea and 0.125 *M* NaCl. They were subsequently washed three times with a cold, buffered, isotonic NaCl solution. The fertilized eggs thus treated still exhibited normal cell division when returned to sea water, but the cell membranes were more friable and the eggs could be broken down by forcing them through the syringe three times. When centrifuged at a low temperature, a cell-free particulate system was obtained from the fer-

tilized Arbacia eggs which gave phosphorylation ranging up to 30 per cent ester formation from the inorganic phosphate present when succinate was used as substrate and over 20 per cent with glutamate and α-ketoglutarate. The results obtained with the substituted phenols, although not yet extensive, indicate that the effects on phosphorylation in the fertilized and unfertilized egg extracts are identical.

The concentrations of 4,6-dinitrocresol and 2,4,5-trichlorphenol required to give a 50 per cent block to phosphorylation of the cell-free homogenates obtained from unfertilized and fertilized Arbacia eggs were identical within the range of experimental error, being 7.9×10^{-7} *M* and 5.6×10^{-6} *M*, respectively, for homogenates from unfertilized eggs and 10^{-6} *M* and 4×10^{-6} *M*, respectively, for the homogenates obtained from fertilized eggs at pH 7.2.

Similar experiments were started with unfertilized and fertilized eggs of *Mactra solidissima*, which have extremely tough membranes. On account of lack of material, further experiments on Mactra have been postponed until next season.

Action of a series of nitro- and halophenols on the respiration and cell division of Mactra solidissima. A. K. Keltch, Philipp Strittmatter, C. P. Walters and G. H. A. Clowes.

A series of nitro- and halophenols which block cell division and stimulate respiration of Arbacia also block cell division and stimulate respiration of Mactra, while those that are inactive on Arbacia are also inactive on Mactra. This block to division is, as in the case of Arbacia, fully reversible over a wide range of concentrations. The concentrations of nitro- and halophenols required to effect a fifty per cent block to cell division were generally four to eight times the concentrations required to exert similar effects on Arbacia. The concentration of substituted phenol which gives the maximum increase in respiration corresponds very closely with the concentration which produces the initial cell division block.

The respiratory stimulation effected by these nitro- and halophenols on Mactra is, when expressed as per cent increase over untreated eggs, double the increase effected by the same compounds on Arbacia. For example, 2,4-dinitrophenol increases respiration of (a) fertilized Arbacia eggs 290 per cent and (b) fertilized Mactra eggs 650 per cent.

The experimental procedure followed was the same as that used for Arbacia eggs (*J. Gen. Physiol.*, 1936, **20**: 145) except that the substituted phenols were added to the eggs at 40 minutes after fertilization instead of 25, thereby allowing time for maturation of the Mactra eggs. The effects exerted by a series of substituted phenols on the respiration and cell division of Mactra are reported in the accompanying Table.

Conc. reagent	2,4-dinitrophenol		4,6-dinitrocresol		4,6-dinitrocarvacrol		2,4-dinitrothymol		2,4,5-trichlorophenol	
	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg
Moles per l.										
0	63	3.6	64	3.6	65	3.7	55	3.8	58	3.7
1×10^{-6}										
2×10^{-6}							100	3.8	67	3.7
4×10^{-6}					124	3.7	113	3.8		
8×10^{-6}	81	3.5					162	3.0	94	3.7
16×10^{-6}	96	3.6	169	3.6	243	3.7	95	0.0	117	3.7
32×10^{-6}	104	3.6			314	2.5	77	0.0	220	3.7
64×10^{-6}	161	3.6	349	0.3	312	0.0	23	0.0	450	0.0
128×10^{-6}	218	3.6	436	0.0	224	0.0	20	0.0	138	0.0
256×10^{-6}	320	3.4	410	0.0	140	0.0	13	0.0	115	0.0
512×10^{-6}	407	0.6								
1024×10^{-6}	400	0.0	116	0.0	73	0.0			88	0.0

Action of dinitrocarvacrol and dinitrothymol on respiration of fertilized Arbacia and Mactra eggs and certain mammalian tissues and cell-free, particulate, phosphorylating systems. G. H. A. Clowes, A. K. Keltch, Philipp Strittmatter and C. P. Walters.

The effects exerted by varying concentrations of dinitrocarvacrol and dinitrothymol on the respiration and cell division of fertilized Mactra eggs are presented in the last two sections of the Table in the preceding paper. In both cases a reversible block to cell division is effected at a given concentration of the reagent and a peak of respiration is observed at or just beyond the highest concentration at which cell division still occurs. These effects are in sharp contrast to those observed with Arbacia. While dinitrocarvacrol induces in fertilized Arbacia eggs the usual marked rise in respiration, reaching a peak at a point corresponding approximately with that at which a 50 per cent block to division is induced, dinitrothymol, while giving a reversible block to cell division in the same manner as dinitrocarvacrol, exerts no stimulating effect whatever on respiration, but induces a sharp fall in oxygen consumption from the beginning.

The cell-free, oxidative, phosphorylating extracts of unfertilized Arbacia eggs give results corresponding with those obtained with the intact fertilized eggs. Both dinitrocarvacrol and dinitrothymol induce reversible blocks to phosphorylation at concentrations corresponding with those required to block cell division. Dinitrocarvacrol, with rising concentration, causes a slight increase in respiration, followed by a fall, while dinitrothymol, with rising concentration, causes an abrupt reduction in respiration, as in the case of the intact eggs.

Unfortunately, we have not thus far had an opportunity to test cell-free, particulate systems derived from Mactra eggs. Cell-free, particulate systems derived from mammalian kidney and liver exhibit with both isomers a block to phosphorylation, with a corresponding slight, temporary rise in respiration.

From previous experiments the conclusion has been reached that adsorption of the un-ionized substituted phenol molecule on the surface of the oxidative, phosphorylating enzyme is probably responsible for the reversible block to cell division and the corresponding fall in respiration in fertilized Arbacia eggs. Some evidence has been presented indicating that the initial stimulation of respiration may be attributable to the substituted phenol ion. The experiments reported above support this viewpoint. The fact that a series of nitro- and halophenols block cell division and respiration at far lower concentrations in Arbacia than in Mactra suggests that components of the surface of the phosphorylating enzyme in Arbacia have a greater affinity for the un-ionized substituted phenol molecules than do corresponding components of the surface of the phosphorylating enzyme in Mactra. The peculiar behavior of dinitrothymol in depressing respiration in Arbacia eggs and cell-free, phosphorylating systems derived from these eggs, while stimulating respiration in Mactra eggs and in cell-free mammalian kidney and liver phosphorylating systems, may well be attributable to steric hindrance due to the presence of the isopropyl group in the ortho position to the phenolic hydroxy group in dinitrothymol. This might interfere with the action of the ion, particularly in the case of Arbacia, in which the affinity of the un-ionized molecule of the substituted phenol for components of the surface of the Arbacia phosphorylating mechanism is presumed to be greater than that for the corresponding components of the Mactra phosphorylating system.

The complete reversibility of the cell division block in both Arbacia and Mactra, even after exposure of the eggs for a considerable period to concentrations 1000 fold those required to block division, lends support to the view that the attachment of the un-ionized substituted phenol molecule to surface components of the phosphorylating enzyme is probably due to hydrogen bonding and van der Waal effects.

Some observations on phosphate uptake by Tetrahymena geleii. Jay S. Roth, Alfred M. Elliott and Robert L. Hunter.

Tetrahymena geleii E was grown in a synthetic medium containing 100γ of phosphate labeled yeast nucleic acid per ml. of medium. The labeled nucleic acid was prepared by growing yeast in the presence of P^{32} using a modification of the method of DiCarlo (*J. Biol. Chem.*, 1949, **180**: 329). The only other source of phosphorus was protogen, an essential growth factor for the protozoa which contributed 2.04γ of phosphorus per ml.

The uptake of phosphorus from the yeast nucleic acid was determined by following the change in activity of the supernatant and in the washed protozoa. Of 7.65γ of nucleic acid phosphorus per ml., 3.52γ or 46% was taken up during the first 60 hours. The rate of uptake was constant from 12–60 hours and became zero after that time. The number of organisms reached a maximum at 60 hours. Inorganic phosphate in the medium was also followed and found to increase during the first 24 hours of growth, followed by a slow decline. This gives further support to the observations that *Tetrahymena geleii* produces an enzyme capable of splitting phosphate from nucleotides or nucleic acid, into the medium.

Double refraction of flow studies on Desoxypentose nucleic acid. ALEXANDER RICH.

Until recently, the double refraction observed in solutions subjected to a velocity gradient could be used to measure particle size only for the limiting case of extinction angle approaching 45°, *i.e.*, at low velocity gradients (*Adv. in Coll. Sci.*, 1942, **1**: 269). It is now possible to make measurements for all values of the extinction angle and translate these results into particle size (Sheraga, Edsall and Gadd, to be published). By this means, one can ascertain whether a given solution acts as a mono- or polydisperse system. In addition, the influence of concentration can be studied, thereby obtaining a measure of molecular interactions.

In this study, the desoxypentose nucleic acids (DNA) were prepared by the method of Gulland *et al.* (*J. C. S.*, 1947, **11**: 29) from the testis and spermatophores of the squid, *Loligo pealii*. The N/P ratio was 1.70. All double refraction of flow (DRF) experiments were carried out at 24°C.

The effect of concentration on the apparent rotary diffusion constants (θ) of DNA was studied in a 2 *M* NaCl solution. At concentrations of .15% DNA, the deviations of the extinction angle-velocity gradient curves from that expected of a monodisperse, non-interacting solution were marked. However, on reducing concentration to .06 or .04%, the curves rapidly approached the theoretical relationship for a monodisperse system. This indicates that the DNA preparation is homogeneous as respects particle length in the region studied for velocity gradients up to 500 $sec.^{-1}$.

An attempt was made to calculate the rotary diffusion θ for infinite dilution by extrapolating to zero concentration. This could not be done directly, since the change in θ becomes very large at low concentrations. θ is inversely proportional to an inner frictional constant ζ, which is a measure of the torque required to maintain the molecule rotating with unit angular velocity. It was expected that ζ should decrease with a fall in concentration, and on plotting concentration vs. $1/\theta$ a straight line was obtained.

The remarkable linearity of concentration and $1/\theta$ is equivalent to stating that the inner frictional constant ζ is composed of an intrinsic part (ζo) and a concentration dependent portion (ζc), *i.e.*, $\zeta = \zeta o + \zeta c$. The slope of this line is an interaction constant

$$\left[\frac{d\left(\frac{1}{\theta}\right)}{dc} \alpha \frac{d\zeta c}{dc} = K\right]$$

characteristic of the parameters of the solution, *i.e.*, pH, ionic strength, etc., and can be used as a quantitative measure of molecular interaction.

On extrapolating to infinite dilution, DNA in 2 *M* NaCl has a rotary diffusion constant of 72, and a particle length of 6650 A° (for axial ratio = 200).

The kinetics of denaturation of several proteins. ROBERT C. WARNER AND MILTON LEVY.

The kinetics of denaturation of several proteins have been examined as a function of pH and temperature. Denatured protein was determined as that material which became insoluble at the isoelectric point of the protein. Although there are numerous reports in the literature of first order denaturation reactions, and first order kinetics were found for the denaturation of ricin (*J. Biol. Chem., In press*), only ovalbumin of the four proteins studied proved to be of this type. Bovine serum albumin, lactoglobulin and horse methemoglobin all showed characteristics of higher order reactions.

The data for ovalbumin are most simply interpreted on the assumption that the protein consists of two components which denature at different first order rates.

Serum albumin denaturation is characterized by a lag period during which no insoluble protein is formed, followed by the formation of precipitable protein approximately by a second order process. The entire reaction is best fitted by an initial first order reaction leading to the formation of a soluble intermediate which then polymerizes to give an insoluble product. The slope of a second order plot is proportional to the initial protein concentration at alkaline pHs, but in acid solution a greater dependence on initial concentration is found.

Lactoglobulin shows the same sort of kinetics as serum albumin in acid solution, but the lag period disappears at alkaline pHs. The slopes of the second order plots are dependent on the initial protein concentration.

Horse methemoglobin denaturation is best fitted by a second order plot without a lag period. The experiments were all done at a single protein concentration.

For consideration of the variation of rate with pH the second order slopes were compared at a constant initial protein concentration.

The effect of pH upon the rate of denaturation of several proteins. MILTON LEVY AND ROBERT C. WARNER.

By working at constant protein concentration, in those cases where this has a significant effect on rate, psuedo constants have been obtained for a wide range of pH values for the rates of denaturation (insolubilization) of bovine serum albumin, horse methemoglobin, lactoglobulin and ovalbumin. These constants when plotted for a single temperature as logarithms against the pH show that only a few acid-base groups are significant in determining the rates in spite of the many such groups present in the proteins. Bovine serum albumin shows minimal rates at pH 2.5 and 6.5 at 66° with a very high intervening maximum centering at pH 4. Between pH 2.5 and pH 4 the temperature coefficient of rate is very small, whereas at all other pH values it is large. The high maximum at pH 4 could explain the finding (attributed to reversibility) that solutions of serum albumin heated in acid solution and neutralized while hot, precipitate completely; whereas those which are first cooled to room temperature and then neutralized are nearly completely soluble. The data tend to confirm the hypothesis that prototropic hydrogen bonds are much more important elements in the structure of native proteins than are salt bridges formed between positive and negatively charged groups by virtue of coulombic forces.

Some physical constants of the Fundulus egg. OTTO GLASER.

An adequate back log of physical data on developing *Fundulus heteroclitus* is difficult to find or establish. Even after mechanical or chemical removal of the outer "jelly," fibrous and sticky, weight/volume ratios without further correction may be irreconcilable with the fact that, in sea water, these eggs invariably sink.

Absurdities are completely eliminated by the flotation method. Four volumes of sea water plus one volume M sucrose can be easily adjusted so that the eggs are just afloat. Density is then given by the Westphal balance. Thirty-six experiments averaged a floating density of 1.0464 for the capsules with eggs but without jelly. This value averages 2.25% more than the average of the corresponding sea waters. Within datum, we can approximate closely the average flotation densities without flotation.

Our chief interest is the egg inside the capsule. Both, we assume, are spheres. Frequently this agrees with the perpendicular diameters measured by ocular micrometer; sometimes the assumption is only the simplest approximation.

The difference between the two volumes is the volume of the highly variable perivitelline space. If perivitelline liquid is essentially sea water, the density of the latter enables us to calculate the weight of the intracapsular fluid. Subtraction from total capsular weight, known by flotation or direct weighing, gives us the weight of the egg proper. Egg-densities average 1.0537. An average based only on extremes comes to 1.0530. Egg weights vary from 2.38 to 3.47 mgs.; volumes, from 2.26 to 3.30 mms^3. Densities based on these extremes average 1.0523. Average egg-densities during the first four days of development are 0.0063 greater than during the days just prior to hatching. We may doubt if this difference is significant.

Temperature alteration of carbamate narcosis of dividing Arbacia eggs. IVOR CORNMAN.[1]

The amount of ethyl carbamate (urethan) necessary to produce 20–40% retardation of Arbacia egg cleavage varies with temperature. At 12°, 16° and 20°C., the effective concentrations of urethan are 2.5, 5 and 6.1 g./L, respectively. At 21°, 25° and 29°C. the doses are 7, 5, and 4 g./L. Urethan is least effective at 20°–21°C., and becomes more effective if the temperature is raised or lowered beyond this point.

These opposing trends indicate that two mechanisms are involved and that urethan acts upon one or both to produce the same visible result. Urethan narcosis both at high and low temperature is characterised by inhibition of cytoplasmic cleavage while the nuclear processes continue and eventually produce four blastomeres by means of two simultaneous cleavages. Increased effectiveness at high temperatures is consistent with the proposal that urethan facilitates denaturation of protein. Chemical combination has been proposed as a mechanism by which urethan exerts some of its effects. Combination would be enhanced at low temperatures and so could explain the increased narcosis below 20°C. A probable site of combination on the urethan molecule is the amino group, but when one or both of the amino hydrogens are replaced with alkyl groups, the effectiveness of the carbamate is increased. Ethyl-N,N-diethyl carbamate, $(C_2H_5)_2NCOOC_2H_5$, is four times as potent as ethyl carbamate, but like ethyl carbamate is least effective at 20°–21°C. and selectively inhibits cytoplasmic cleavage. Propyl and butyl carbamate are also more active than ethyl carbamate. Inasmuch as substitution on either or both ends of the urethan molecule augments its inhibition of cleavage, the amino or ester linkages appear to be unlikely sites for the narcotic to combine with cellular components. Adsorption of the intact molecule appears more probable.

Isotonic solutions for the erythrocytes of the smooth dogfish, mackerel and tautog. JAMES W. GREEN AND JOSEPH F. HOFFMAN.

As an outgrowth of studies on the permeability of fish red cells an investigation was made to determine what concentrations of salt solutions were isotonic with the red cells of the smooth dogfish, mackerel and tautog. Previous work in this field had been chiefly confined to freezing point depression measurements of whole blood or serum and the calculation from such values of isotonic solutions. Four methods were used in the present study for the determination of isotonicity: (1) freezing point depression, (2) hematocrit, (3) volume changes by densimeter, and (4) hemoglobin concentration changes. In general the isotonicity of each sample of blood was determined by each of the 4 methods. Blood was removed from the caudal vessels of the smooth dogfish by syringe, from the mackerel and tautog by cutting the gills or by heart puncture. For tautog and mackerel it was necessary to pool the blood obtained from several fish. Both oxalated and defibrinated blood was used. The isotonic concentrations were the same from blood drawn from gills or heart which indicates that negligible amounts of sea water were obtained with gill blood.

The following average isotonic values were obtained for NaCl expressed as moles. The measure of variation is shown by the standard error.

Fish	Hematocrit	Hemoglobin	Densimeter	ΔT_f
Dogfish	0.34±.008	0.35±.02	Ca. 0.53	0.57±.003
Mackerel	0.27±.007	0.26±.005	0.26±.004	0.33±.004
Tautog	0.21±.007	Ca. 0.23	0.26±.007	0.27±.008

Further dogfish red cell studies were made with NaCl-urea solutions (22.28 gm. NaCl and 21.4 gm. urea per liter), calculated as 0.56 *M* NaCl. On this basis 0.47 *M* ± .004 was isotonic

[1] Aided by a grant from Bache Fund of the National Academy of Sciences and by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University.

by the hematocrit; 0.51 ± .009 isotonic by hemoglobin analysis and 0.52 ± .005 isotonic by the densimeter.

Further studies on the action of a cytoplasmic component on jelly coat and membrane formation in Arbacia punctulata. ALBERTO MONROY AND JOHN RUNNSTRÖM.

As a continuation of previous work of the present authors (*Ark. f. Kemi*, 1950, 2) the precipitation of the jelly coat of intact eggs under the influence of egg extracts has been investigated. 1 *M* KCl extracts of frozen dried eggs of *Arbacia punctulata*, dialysed against sea water, have a strong precipitating action on the jelly coat both *in vivo* and *in vitro*. The extracts were fractionated by ultracentrifugation and the separated fractions controlled electrophoretically. After removal of a first fraction sedimenting at 25,000x g, ultracentrifugation at 155,000x g leads to the formation of two layers: a heavily pigmented sediment and a slightly yellowish supernatant. The activity is entirely confined to the sedimented fraction. In this latter two components can be recognised on electrophoresis. Attempts at further purification have so far failed. Thrombin has also been found to be active in precipitating the jelly coat, both *in vivo* and *in vitro*. This, however, occurs only when the salt concentration of the medium is lowered. The effect of thrombin is completely counteracted by heparin. The cytoplasmic component isolated according to the procedure indicated above corresponds to the "antifertilizin" (AF) of the egg. AF inhibits the agglutinating action of "fertilizin." It was often observed that AF not only precipitates the jelly coat but also renders the vitelline membrane more refractive. Eggs were fertilized in an 0.0026 *M* solution of Jodoso-benzoic acid in sea-water. The membranes formed soon become more refractive than normally. An increased retardation can be demonstrated in the polarization microscope. Gradually also a coagulation of the surrounding jelly coat may occur. These changes are correlated with an increased release of pigments to the perivitelline space. Observations on previously centrifuged eggs show that the active agent is released from the pole containing the pigment granules. Our data when considered together suggest that AF is released from the pigment granules, subsequent to the attachment of the spermatozoon, and that it exerts a *coagulating* action on the fertilization membrane.

The exchange of radioactive calcium by unfertilized and fertilized Arbacia punctulata eggs with or without jelly coats. F. HERMANN RUDENBERG.[1]

During measurement of calcium uptake by sea urchin eggs, designed to unearth any temporal correlation which may exist with observed structural changes, a considerable difference in the amount of calcium taken up by eggs with or without jelly coats has been observed.

The experimental solution surrounding the eggs was sea water containing a small amount of radioactive calcium (maximum, 1.05×10^{-2} mg. or 0.52 μc per ml.). Uptake, by the eggs, was followed by measuring the disappearance of the isotope from the surrounding sea water. The experimental eggs were crowded (up to 3.5×10^{6} per 10 cc.) in the bottom of a dish but constant agitation permitted them, when fertilized, to develop as quickly as dilute (10^{3} per 10 cc.), non-agitated control eggs in non-radioactive, calcium-enriched sea water and in normal sea water (pH 8.2–8.4). There was no pH change in any dish and the temperature remained at 20.0°C. At least 98% fertilization was obtained in accepted experiments.

Eggs with jelly coats show an increase of Ca^{45} before and after fertilization followed by a decrease from the sixth to the tenth hour after fertilization, when hatching occurs. Eggs freed from jelly coats and washed repeatedly show no such increase and subsequent decrease. Eggs with jelly coats which have taken up Ca^{45} and then by gentle shaking gradually have their jelly coats removed, show a subsequent decrease of radioactivity until all the jelly coats are off. There is no measurable uptake of Ca^{45} by denuded eggs when the dissolved jelly coat material is left in the medium in which the eggs are suspended.

The simplest conclusion is that some substance in the jelly coat binds calcium as the jelly coat swells, and loses it preparatory to hatching. At present, however, it is impossible to state what proportion of the Ca^{45} is exchanged and what proportion is accumulated.

[1] Work done during tenure of Atomic Energy Commission Predoctoral Fellowships in the Biological Sciences.

The effect of tissue extracts on cell division. L. V. HEILBRUNN, W. L. WILSON AND D. HARDING.[1]

The appearance of the mitotic spindle and the division of a cell are preceded by a gelation of the protoplasm, the mitotic gelation. In view of the fact that the gelation or clotting of protoplasm is typically a process similar to blood clotting, it is not surprising that the mucopolysaccharide heparin prevents mitotic gelation just as it prevents the clotting of vertebrate blood. This was shown to be true by Heilbrunn and Wilson (1949) and they also showed (1950) that the bacterial polysaccharide of Shear, which is either a heparin or a heparin-like substance, also prevents mitotic gelation and cell division. The bacterial polysaccharide can cause regression of tumors in mice, but it is toxic for men. In view of the fact that heparin-like substances are found in practically all types of living material, we felt that it might be possible to extract from some tissue a mucopolysaccharide which would cause regression of tumors in men as well as in mice.

After various unsuccessful attempts, we found that extracts made with acidified sea water were very effective in preventing the mitotic gelation as it occurs in the eggs of the worm Chaetopterus. These extracts were prepared from the posterior segments of the Chaetopterus, from the clam Mactra, from the ovaries of the sea urchin Arbacia and the sand dollar Echinarachnius; but by far the most potent extracts were obtained from the ovaries of the starfish Asterias. These extracts prevent the mitotic gelation and they also prevent cell division. They also cause a liquefaction of the cortex of the egg.

The extract from the starfish ovary is more potent if the ovary contains few rather than many eggs. The active substance in the extract is heat stable.

The action of heparin on fertilization in the eggs of Arbacia punctulata and Echinarachnius parma. CLIFFORD V. HARDING.[2]

Dilute solutions of jelly coat substance inhibit the normal differentiation of the fertilization membrane (*Exp. Cell Res.*, 1950). It became of interest to test the action of other substances, chemically related to the jelly coat substance, on fertilization. Three samples of heparin (Hynson, Westcott, Dunning; Upjohn; Vitrum) were tested for their action on fertilization and first cleavage (see also P.S.E.B.M., 1949, **70**). Hynson, Westcott, Dunning heparin proved to be the most active and in concentrations of 0.025 per cent effectively altered normal fertilization (the membranes remained very low and granular). Moreover, this heparin had the same effect on membrane formation in eggs which were activated with hypertonic sea water. This suggests that the heparin has its effect primarily on the egg and not the sperm. A concentration of 0.2 per cent heparin completely blocked fertilization in the eggs of Arbacia, and they did not divide. This prevention of cleavage is most likely due to a blocking of fertilization, since eggs placed in heparin after fertilization cleave normally (see A. Gagnon's abstract). Eggs which were treated with trypsin to remove the vitelline membrane, fertilized in sea water, and transferred to 0.2 per cent heparin in Ca-free sea water (to dissolve the hyaline layer) showed high percentages of cleavage. In this case, the external layers were removed to insure penetration of the heparin to the egg surface.

Heparin solutions treated with small amounts of sodium periodate did not prevent membrane elevation or cleavage. Heparin Vitrum and Upjohn heparin were relatively weak in their action on fertilization. However, if the solutions were autoclaved for 10 minutes at 250°F. they became active in preventing membrane elevation in concentrations of 0.025 per cent. Treatment with periodate removed this effect. Fucoidin, a polysaccharide, and the human H substance also inhibited membrane elevation. The activity of these was removed with periodate. These results are of interest in relation to the work of Runnström and Kriszat.

[1] Aided by a grant from the U. S. Public Health Service.
[2] National Institutes of Health post-doctorate fellow.

The action of heparin on fertilization and cleavage in some marine eggs. ANDRÉ GAGNON.[1]

Earlier work has shown that heparin tends to prevent both fertilization and cleavage.

The eggs of *Mactra solidissima, Arbacia punctulata,* and *Echinarachnius parma* were studied. A 0.2% heparin solution (Hynson, Wescott, and Dunning) made up in filtered sea water was used in all the experiments.

In one series of experiments, the eggs were placed into the heparin solution at various intervals before fertilization, then fertilized and allowed to develop in it. With Mactra eggs thus treated, there results a high inhibition of cleavage (as high as 92%). Treatment of Arbacia eggs under similar conditions gives even higher cleavage inhibition (average 95%). The results also indicate that the time of exposure of the eggs before fertilization does not affect the final high percentage of inhibition. With the Echinarachnius egg, preliminary work indicates that there is a delay, but that ultimately cleavage is almost the same as in the controls. In other experiments, the eggs were fertilized in sea water and then exposed to the heparin solution at various intervals after fertilization. The following results were obtained. With Mactra there is some inhibition, and the period of sensitivity is restricted to a short period following the addition of the sperm. In Arbacia and Echinarachnius, there is essentially no inhibition. From these results, it is concluded that the main step affected in Arbacia and Mactra is fertilization. In Arbacia, eggs that are fertilized in heparin lack a fertilization membrane (see C. Harding's abstract). In Chaetopterus, Heilbrunn and Wilson have found that sperm penetration and fertilization may occur in the presence of heparin; yet, there is inhibition of cleavage.

An experimental study of maturation in Chaetopterus eggs. LESTER GOLDSTEIN.

Since eggs of *Chaetopterus pergamentaceus* apparently mature as a result of leaving the inhibiting environment which surrounds the ovary, and not by any stimulus provided by the sea water environment, an attempt was made to find what substance, or substances, was inhibiting maturation *in vivo*. Experiments utilizing sea water saturated with carbon dioxide showed that one of the inhibitors could very well be carbon dioxide. Carbon dioxide solutions would completely inhibit maturation and reversibility was also complete when the eggs were returned to normal sea water. Though carbon dioxide causes sea water to become acid, it was demonstrated, over a wide range of pHs, that the maturation process was not affected by any pH value in itself. Consequently, the inhibition is probably due to a specific action of carbon dioxide.

While carbon dioxide, which may accumulate in the ovaries due to the respiration of the eggs, may be an inhibitor, it probably can account for only about 50 per cent of the inhibition within the animal. Another inhibiting factor may exist *in vivo* and preliminary experiments indicate that this is possibly a protein or a compound bound to a protein.

Other experiments, utilizing sodium citrate, show that calcium ions are necessary for maturation to proceed. This calcium is necessary in or on the eggs, since maturation will proceed normally in environments lacking calcium.

Changes in the protoplasmic cortex during cell division. WALTER L. WILSON.[1]

During the course of cell division, changes occur in the colloidal state of the cell cortex. In the egg of the annelid worm, *Chaetopterus pergamentaceus*, it is possible to make a physical study of the protoplasmic cortex. For in this egg, granules can move from the cortex under moderate centrifugal force, and the force necessary to dislodge the granules varies during the mitotic cycle.

In the present work, eggs were subjected to various centrifugal forces at intervals between fertilization and cleavage and the cell cortex examined under high power magnification to determine whether or not the granules were present in the cortex. Shortly after fertilization (at 21.0°C.) the rigidity of the cortex decreases. After several minutes the rigidity of the cortex increases again. At about 10 minutes after fertilization the cortex has returned to its original

[1] Aided by a grant from the U. S. Public Health Service.

rigid condition and remains so until about 35 minutes after fertilization, at which time there is a decrease in the cortical rigidity. The cortex remains in this condition until the cell divides. Thus the cell cortex becomes rigid prior to the gelation which is known to occur in the cell interior. Experiments carried out while the cleavage furrow was forming show that there is no increase in the rigidity of the cortex at this time.

Microscopy in the ultraviolet visible and infrared. GEORGE I. LAVIN.

In 1946 a description of a set-up (*Science*, 104: 61) was published whereby photomicrographs, using various wavelengths of light, could be obtained. It was found that this arrangement was rather cumbersome in use. In the present ensemble the microscope and camera are placed on the top of a small table. The quartz mercury resonance lamp is mounted directly underneath the table top. The light from the lamp reaches the microscope through a hole bored through the table.

With this source and a cobalt sulfate nickel sulfate filter the 2537A° mercury line can be isolated and used to image and focus a field with a willemite screen. The 5460A° line, from the same source, can be obtained with a Wratten 77A filter. This can be focussed with a ground glass screen. The light from a tungsten lamp is introduced by means of a right angle prism, which can be thrown in and out of the field. A Wratten 25A filter is used to obtain a field and focus after which an 88A filter is used in conjunction with infrared sensitive plates.

Preliminary results indicate that different structures are revealed by use of the various regions of the spectrum. The method has been applied to a study of Arbacia eggs and plutei.

Invertebrate photoreceptors. LORUS J. MILNE AND MARGERY J. MILNE.

A review of the extensive and widely scattered literature on light perception among invertebrate animals has been made to furnish a more integrated basis for future research in this field, along both anatomical and physiological lines. The range of photosensory mechansims is far more impressive than that to be found among the vertebrate series, and analogous mechanisms appear to have arisen independently in very many places. Perhaps the most distinctive feature of the survey has been the widespread and important reliance of invertebrates upon dermal light sensitivity without distinctive sensory structures, and their dependence upon such systems for interpretations of presence or absence of light, changes of intensity (whether sudden illumination or shadowing), of direction of light source, or spectral content. Often behavior studies have indicated far more excellence in light perception than would be predicted from structural investigations. Correspondingly, the supplementary action of dermal sensitivity in many instances renders difficult the interpretation of vision by more highly complex photosensory structures. Among the polychaete annelids, the gastropods, pelecypods and cephalopods, and certain arachnids, useful image formation appears to be provided for—often with mechanisms allowing accommodation as well as the more widespread adjustments in sensitivity with dark or light adaptation. In a few polychaetes, gastropods, and crustaceans and insects, compound eyes furnish the basis for mosaic vision with varying degrees of resolution and sensitivity. Embryological information and details of degeneration of visual mechanisms in parasitic, abyssal, speleologic and fossorial species have provided further understanding of the inter-relationships between functional photoreceptors.

The loci of action of ultraviolet and x-radiation, and of photorecovery, in the egg and sperm of Arbacia. HAROLD F. BLUM, J. COURTLAND ROBINSON AND GORDON M. LOOS.

Delay of cleavage of Arbacia eggs by ultraviolet radiation (λ 0.27μ to 0.313μ), is associated with the nucleus: The cleavage of enucleate halves is not delayed if these are irradiated before fertilization. But if the sperm nucleus is introduced by fertilizing enucleate halves before irradiation, or if the sperm itself is irradiated before fertilization, delay occurs. Nucleate halves, like whole eggs, show delay of cleavage under any of the above conditions. Thus, only when a nucleus (of either egg or sperm) is exposed to ultraviolet radiation does delay result.

Photorecovery after exposure to ultraviolet radiation is associated with the cytoplasm of the egg: Illumination of sperm with visible radiation ($\lambda \backsim 0.4\mu$ to 0.5μ) after exposure to ultraviolet, does not diminish the delay of cleavage of eggs fertilized with this sperm. If eggs are illuminated after fertilization with ultraviolet irradiated sperm, photorecovery is observed. This is also true for nucleate and enucleate halves when treated in the same way. Photorecovery occurs in whole eggs, nucleate, or enucleate halves when fertilized with normal sperm and then exposed to ultraviolet radiation. When eggs or nucleate halves have received ultraviolet radiation before fertilization with normal sperm, photorecovery is observed. Under such conditions, photorecovery cannot be demonstrated in enucleate halves, there being no delay of cleavage. Thus, photorecovery occurs in whole eggs, nucleate or enucleate halves, but not in sperm. Sperm and egg are comparable here to bacteriophage and *E. coli* in Dulbecco's experiments.

Similar experiments with x-radiation relate the locus of cleavage delay to the nucleus. No photorecovery was demonstrated in the experiments with x-rays, suggesting a fundamental difference in mechanism of action from that of ultraviolet radiation.

Integumentary pigmentation and the coelomic fluid of Thyone Briareus (Lesueur). NORMAN MILLOTT.

The black color of Thyone is due to an integumentary pigment occurring either as free granules or within cell bodies, some of which resemble degenerating amoebocytes of the type found in the coelomic fluid.

The pigment shows the chemical properties characteristic of melanin (Lison, 1936). The participation of the amoebocytes of the coelomic fluid in pigment formation is at once suggested by the fact that when the coelomic fluid is exposed to air it throws down a clot, including many amoebocytes, which slowly changes from white through yellow to brown, and eventually breaks up into irregular globular masses. These strongly resemble the black bodies found normally occurring in the perivisceral coelom. The depth of color of the clot can be increased by addition of a trace of catechol to the coelomic fluid *in vitro*. Coelomic fluid *in vitro* will oxidize other phenols such as "dopa," pyrogallol and p-amino-phenol at pH 7.4, 7.6 and 30°C. Only a few samples oxidised guaiacol, and tyrosine is slowly oxidised at pH 7.0 and 30°C. only when a trace of catechol is added to the substrate. These actions are slow and feeble, requiring 9–20 hours in order to produce a distinct color reaction. They are abolished by boiling the coelomic fluid or adding 0.0005 *M*–0.001 *M* cyanide. Whether these oxidations are truly enzymatic must remain uncertain. The coelomic fluid shows a well marked positive oxidase reaction with α-napthol and paraphenylene diamine (Snell and Snell, 1937), but it is not attributable to enzymes, being unaffected by 0.001 *M* cyanide and by boiling for 30 minutes. Possibly the action may be due to substances such as orthoquinones (Pinhey, 1930; Wheldale-Onslow, 1931).

No indication has yet been obtained of the substrate on which these substances may act *in vivo*, but it is significant that spectophotometric examination of the centrifuged coelomic fluid revealed no tyrosine in either amoebocytes or the fluid itself. Similarly, no color reaction was given with Millon's and Morner's reagents, although a feeble positive reaction was shown with Folin and Denis' reagent.

The feeble oxidation of phenols cannot be due to a low oxidation-reduction potential, for E_o' for the coelomic fluid in equilibrium with air at pH 7.0, estimated colorimetrically, is $+0.218-+0.224$. Similarly there is no evidence of an interfering dehydrogenase, for methyl alcohol does not increase the rate and extent of darkening of the coagulum in the coelomic fluid *in vitro*, nor do dehydrogenase inhibitors such as acetone, chloroform and phenyl urethane appreciably affect the reaction with α-napthol and paraphenylene diamine.

In order to ascertain whether amoebocytes carrying effete substances will deposit them in the pigment bearing areas, iron saccharate in sea water was injected into the perivisceral coelom. Amoebocytes were observed to take up the saccharate, some of which was eliminated via the cloaca, but further details must await the preparation of sections. It may be mentioned that methylene blue injected into the perivisceral coelom in connection with oxidation-reduction studies, was seen to be eliminated rapidly by the gut, respiratory trees and bodywall. Both active transfer by the gut wall and the activities of amoebocytes are involved, the latter, on occasion, being so active in elimination that the coelomic fluid becomes almost devoid of them. That many amoebocytes are lost via the cloaca may be shown by inserting a plug, when it is seen

that not only a large measure of dye but also many of the amoebocytes are retained in the coelomic fluid. It is noteworthy that the amoebocytes tend to deposit granules of dye in those areas of the respiratory trees already bearing accumulations of black and brown pigment; and further, that the dye often appeared red in these areas, owing to metachromasy.

Micro-injection of $CaCl_2$ into the giant nerve axon of the squid. ROBERT CHAMBERS AND CHIEN-YUAN KAO.

In micro-operations, it has been general experience that inorganic salts of common monovalent cations liquefy, while those of divalent cations coagulate protoplasm. The cells which show these phenomena are frog muscle fiber, sundry Echinoderm eggs, protoplast of plant cells, (root hairs of Limnobium), and protozoa (amoeba and Stentor). Recently, Hodgkin and Katz (*J. Exp. Biol.*, 1949) reported that the axon material of the giant nerve of the Squid reacts in a different way from that stated above. We have confirmed their results, although not agreeing with the claim of their reference on Stentor. In our experiments we used long tapering micropipets with diameters 30 micra across at 600 micra from the tip which measured 4 micra across. The micropipets were thrust diagonally into the interior of the axon with minimal injury indicated by the maintenance of a colorimetric pH of ca. 6.8 and by the ovoid shapes assumed by oil drops deposited within. Injected solutions of NaCl, KCl and water caused no change whereas, with divalent cations, the oil drops rounded up indicating a fluid environment. The effects were almost immediate for Ca and Sr, and delayed for Mg and Ba. The oil drops appeared always coated with a structureless film which prevented union of the drops when in contact. This film was more evident before than after injection of $CaCl_2$. Sodium alizarin sulfonate, introduced after $CaCl_2$, caused a precipitate whereupon the axon material again gelated but the oil drops were distorted irregularly indicating that the jelly had lost the linear arrangement characteristic of the normal fiber.

Some low frequency characteristics of axoplasm and the nerve membrane. OTTO H. SCHMITT.

Measurements of membrane characteristics by the complex attenuation method in the range 40–10,000 c.p.s. on normal squid nerve emphasize the existence of at least three separate electrical components, one relatively stable and probably associated with physical structure, a second directly related to ionic permeability, and a third which is highly responsive to physiological state. The stable component is responsible for the familiar 1 mfd./cm.^{2} capacitance and has a relatively small temperature coefficient. The permeability term is closely associated with excitability but has an unpredicted frequency minimum. The unstable susceptance component is highly temperature sensitive and responds sharply to ionic changes in the environment.

The volume resistivity of squid axoplasm in the intact axon has been studied as a function of temperature, frequency, and fiber size by a method which permits correction for the conductivity of solution clinging to the outside of the nerve. The mean conductivity value found is 22.5 millimhos cm. at 20°C. or about 51% of the conductivity of Woods Hole sea water. Conductivity changes almost precisely as does that of appropriately diluted sea water in the range 5° to 30°C. No detectable variation of resistivity with frequency is found above 500 c.p.s. and a slight measured variation in the 100 c.p.s. region is probably a by-product of the rapidly changing membrane impedance in this frequency range. No systematic variation of axoplasmic conductivity with fiber size has been noted.

Progressive changes in the nerve membrane admittance after dissection. PETER A. STEWART.

The time changes in the membrane conductance and susceptance of the squid giant axon have been determined by the complex attenuation method in order to establish the normal process of deterioration in an isolated nerve fiber. It is found that the electrical state of the membrane remains almost constant for two or three hours if the nerve is frequently bathed with sea water. This steady state is characterized by full excitability, constant susceptance, and a constant membrane conductance of about 1 millimho/cm.^{2} (at 100 c.p.s.). After two or three hours, the con-

ductance begins to rise, increasing linearly with time for several hours. Excitability is lost when the conductance reaches about 4 millimhos/cm.[2]

If the nerve is not bathed, the conductance rises continuously, no steady condition is observed, and excitability is again lost at a conductance of about 4 millimhos/cm.[2] In both these cases, the susceptance changes relatively little, although a gradual decrease with time is observed at frequencies above 500 c.p.s.

All these changes seem to be completely irreversible and inescapable. Changes produced by experimental agents such as temperature or chemicals are superimposed on this general pattern.

Washing the nerve with potassium-free saline delays the rise in conductance and loss of excitability by an hour or two, but does not change the general pattern. The effect of washing with a large excess of potassium (isotonic KCl) is to eliminate excitability instantaneously and to raise the conductance by a factor of three or more within a few minutes. After this the membrane deteriorates rapidly and the conductance increases to a very high value as the membrane disappears.

Electrically induced optical and dimensional changes in single axons including the squid. Preliminary observations on ion effects. JULIAN M. TOBIAS.[1]

Insofar as the longitudinally oriented e.m.f. impressed upon an axon by the propagating nerve impulse is mimicked by an electrode-applied, longitudinally oriented e.m.f., it is useful to examine changes (electrical, chemical, structural) under and around such polarizing electrodes in an attempt to anticipate what changes may occur at the similarly polarized regions of the propagating impulse.

Platinum electrode polarization of whole nerves or single axon of crayfish, spider crab, *Carcinus maenas*, lobster or frog produces reversible cathodal swelling with increased transparency and increased stickiness whereas anodally one sees shrinkage with increased opacity and increased rigidity. In addition, the single frog axon, but not the others, shows a quick, reversible and fatiguable anodal shortening and subsequent lengthening and marked surface wrinkling on closing and opening the circuit.

These changes are, however, apparently due in part to local electrochemical changes unique for polarizable electrodes (probably pH), since with non-polarizable electrodes (Ag-AgCl or balanced salt wicks leading to calomel half cells) one sees neither the quick axonal "twitch" nor the surface wrinkling in the frog axon, nor the rigidity or adherency changes in any of the axons examined.

With non-polarizable wick electrodes changes are most clearly seen in squid (*Loligo pealii*) giant fibers. There is anodal shrinkage and frequent plasmolysis of the protoplast inside the sheath with marked clouding of the axon. Cathodally there is swelling with excessive transparency. The cloudy material can be moved within the axon by pressure, and when given an opportunity it migrates toward the cathode. These changes are reversible on reversing polarity. Birefringence changes characteristically at the two electrodes. If, however, the axon be polarized with a small pool of isotonic KCl around the anodal wick and a small pool of isotonic $CaCl_2$ or $MgCl_2$ around the cathodal wick then the anode produces increased transparency, the cathode increased opacity.

Preliminary observations on squid axon structure. Light scattering properties using an intracellular light source and mechanical prod. SIDNEY SOLOMON AND JULIAN M. TOBIAS.[1]

To investigate axonal ultrastructure, a method studying light scattering by cellular structural elements (molecular and macromolecular) as a function of activity is being developed. A quartz microneedle, impaling the axon along its long axis, serves both as a mechanical prod and as an intracellular light conduit. So far only white light and magnifications up to 100 × have been used. Only the squid axon has been examined.

[1] This work has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the Universtiy of Chicago.

Three observations of interest have been made: (1) Light emerging from the quartz needle tip and propagated along the long axis of the axon is scattered to produce a typical Tyndall effect. At the magnifications used (100 ×) no otherwise visible granules have been resolved (except as below). This is compatible with the notion that the axoplasm is a colloidal system. (2) More vigorous movement of the prod in the axoplasm produces a local increase in fluidity and the appearance of visible granules. This structural alteration appears to be reversible on standing, but only during the early phases of an experiment. (3) When the prod is slowly and evenly advanced in the axoplasm, a series of alternate, stationary, light and dark, curved bands (concave toward the prod tip) is seen to form in the axoplasm ahead of the tip. These appear to be cup-shaped about the tip and do not seem to extend laterally to include the sheath. Lateral extent may, however, be a function of prod size. Although band spacings and curvatures have been measured and an attempt has been made to correlate these dimensions with the prod tip size and shape, the geometry of the system has not otherwise been controlled.

It is hoped that further investigation will extend and clarify these observations.

Renal chloride excretion by the smooth dogfish, Mustelus canis. RUDOLF T. KEMPTON AND LEONORA BERMAN.

In 51 collection periods on 12 large female smooth dogfish, inulin has been used as a measure of glomerular filtration, and the reabsorption of water, chloride and urea examined simultaneously. Data on the total molecular concentrations as reflected by the depression of the freezing point have not yet been studied.

While the reabsorption of urea from each ml. of filtrate is related to blood level in such a way that a constant concentration of urea is left in the tubule, no such relationship appears with chloride. Instead the reabsorption of chloride from each ml. of filtrate is approximately constant, averaging 10.4 mgm. (expressed as NaCl), with 66% of the values falling between 8.4 and 12.4. Variations are not related to any factor or condition yet uncovered from the data. Water, urea and chloride reabsorption are entirely independent of one another, and there is no evidence of an osmotic control of either urea or chloride reabsorption.

Total chloride reabsorption (mgm./hr./k.) is related to both the volume of filtrate and the amount of chloride filtered. Since the blood level is quite constant, variations in these two factors are similar. Variations in the volume of filtrate appear to be due to varying numbers of active glomeruli, since the chloride reabsorbed from each ml. of filtrate is unaffected by a range of filtration rates in which the maximum is over seven times the minimum. The data suggest that there is a maximum rate of chloride reabsorption somewhere between 30–40 mgm./hr./k. (as NaCl). Artificially raised blood chloride levels will be necessary to establish this point.

Consideration of the U/P ratios leads to the conclusion that relatively minor differences in reabsorption may result in a change from urine in which the concentration of chloride is below blood level to urine in which the chloride is much more concentrated than the blood. Variations in water reabsorption have little determining effect on the U/P ratios. Since an active reabsorption must be invoked to explain the U/P ratio that is below 1.0, the continuity of the data seems to demonstrate that an active reabsorptive mechanism is functioning even when the concentrations are such that the reabsorption is in the direction of a gradient which would permit passive outward diffusion.

Observations on the flight motor of Diptera. EDWARD G. BOETTIGER AND EDWIN FURSHPAN.

Electrical activity not associated with wing movement was detected in the thorax of all (eight) species of Diptera tested, when treated with ether, carbon tetrachloride, and certain other agents to the point of loss of normal reflex activity. These potentials, at a certain depth of anesthesia, are very similar to flight potentials. They are only found in sections of the thorax containing the thoracic ganglion, and simpler, more regular discharges, consisting of a small "end-plate-like" potential from which a full spike grows, may be recorded from the ganglion enclosed in a minimal amount of muscle tissue.

Carbon tetrachloride, in addition to producing large ganglionic potentials, causes contraction of the muscles and small, regular potentials in the absence of the ganglion. Whole flies

under carbon tetrachloride show anesthetic flight and general contraction of the thoracic musculature, which results eventually in cessation of flight due to the wings' being caught in the extreme up or down position (usually up). Then on depressing the posterior end of the scutellum, flight may recur or the wings may "click" into the down position. Dissection of flies under carbon tetrachloride reveals a sudden change in the length of the muscles on passing the clicking point. The path thus travelled by the wings may be strikingly similar to that of normal flight, including the characteristic changes in the angle of attack.

It is suggested that the flight motor, capable of 60,000 cycles per minute in certain midges, depends upon a constant tension-producing excitation of both direct and indirect muscles, initiated by the ganglionic potentials; and that the click phenomenon, resulting from the application of this tension to the peculiar wing articulation, allows a sudden change in the loading of the indirect muscles during flight so as to quick-release the muscles causing the particular movement, and to quick-stretch its antagonist. Resulting tension changes then favor the opposite movement.

Dependence of ciliary action upon acetylcholinesterase activity. GERALD R. SEAMAN.

The ciliate *Tetrahymena geleii* contains an active acetylcholinesterase. The enzyme splits 0.08 μg acetylcholine/hr./mg. dry weight of tissue. The enzyme does not attach butyrylcholine. Eserine (4×10^{-4}M) and DFP (4×10^{-3}M) inhibit hydrolysis of acetylcholine by the enzyme preparation from *T. geleii*. The inhibitors, at concentrations of 3.85×10^{-3}M and higher, inhibit motility of the organism. DFP and eserine apparently inhibit specifically acetylcholinesterase and not energy supplying enzymatic reactions; cells immobilized by inhibitor treatment have the same glycolytic rate and the same rate of oxidation of carbohydrate as do untreated cells. The inhibitions of motility effected by DFP and by eserine are completely reversible by washing.

DFP and eserine also inhibit ciliary activity of the frog esophagus and of the cill of *Venus mercenaria*. The inhibitions in these cases are also completely reversible by washing.

It is concluded that the fibrillar system connecting the base of individual cilium is actually conducting tissue and as such is dependent upon acetylcholinesterase activity.

Microphallid metacercariae encysted in Limulus polyphemus. HORACE W. STUNKARD.

In the summer of 1950, Dr. T. H. Waterman found metacercariae encysted in the eyes of *L. polyphemus*. Examination of a large number of horseshoe crabs disclosed that all of them were infected and larvae were found in the connective tissue of the liver and other organs as well as in the eyes. The metacercariae contained well developed gonads and the seminal receptacles were filled with active spermatozoa. They were fed to baby chicks, half-grown white mice, and young golden hamsters. Excysted larvae were found in the intestine of a white mouse 12 hours after it was fed. Worms recovered from a mouse 4 days after feeding, contained eggs in the uterus. They proved to be microphallid trematodes, similar to and possibly identical with those described by Cable and Hunninen (*Biol. Bull.*, 1940, **78**: 136) as *Spelotrema nicolli*.

Larval trematodes from the planarian, Dugesia tigrinum. HORACE W. STUNKARD.

During the winter of 1949–1950, Mr. J. Louis Bouchard of the Oklahoma A. and M. College found larval trematodes in specimens of *D. tigrinum* obtained from the Marine Biological Laboratory. The worms were referred to me for possible identification. The structure of the larvae indicated that they were azygiids, but no further information was available. Records of the Supply Department of the M. B. L. showed that the planarians were collected from Morse's Pond. Planarians collected from this pond in August, 1950 contained larval worms of the same species and search was made for the snail host of the parasite. Small snails, *Amnicola limosa*, were found infected with the asexual stages of the trematode. Planarians, examined under the microscope and known to be uninfected, were placed in a finger bowl with specimens of *A. limosa*

which were shedding the cysticercous cercariae. After several days exposure, the larval trematodes were found in the pharyngeal pockets of the host, as many as four in a single planarian. The larvae do not encyst or grow while in the planarian, which obviously serves merely as a transfer host. Planarians infected with the larvae have been fed to various fishes.

Notes on growth and cystment in Dileptus binucleatus. ANNA JOSEPHINE BRIDGMAN.

From soil and débris collected in a temporary pool near Chapel Hill, N. C., in 1944, animals identified as *Dileptus binucleatus* have been excysted from time to time. It is only necessary to add tap water to the soil in a finger bowl to find, within twenty-four hours, numbers of these ciliates swimming about.

It was found, after some experimentation, that they could be cultured in a modification of Loefer's culture medium with the addition of generous numbers of Halteria.

Prowazek (1904) briefly described the encystment of Dileptus, attributing it to cold. Studitsky (1930) says that in *Dileptus gigas* it is due to increased salt concentration. Experiments indicate that in *D. binucleatus* it may also be due to decreased salt concentration, since they encyst in distilled water, to lack of food, and perhaps to other unfavorable factors.

The cysts are approximately 60 μ in diameter, have one vacuole, and are slightly yellowish. There are three membranes: an outer clear gelatinous covering which serves to secure them to some solid object; a thick striated mesocyst, apparently non-elastic; and an inner, elastic endocyst.

The excystment process is not essentially different from that described for other ciliates. If the culture fluid in which the cysts are formed is removed and replaced by distilled water or fresh culture fluid the animals will excyst. The beginning of the process is marked by an increase in the size and number of vacuoles. As they increase the animal differentiates, there is considerable movement within the cyst, and the ecto- and mesocysts rupture. The animal, still within the endocyst, bulges out from the original cyst wall, but does not escape. The inner membrane is elastic and stretches considerably at this time, while the outer membranes, still enclosing part of the animal, seem to give very little. The cilia, now well developed, enable the animal to move actively within the cyst, pushing against the endocyst. This squirming may go on for fifteen or twenty minutes. Eventually there is a relatively small break in the endocyst and the animal squeezes through, with the proboscis leading and the rear half of the body somewhat flattened and folded on itself. It swims slowly at first, but soon assumes its usual shape and activity.

The excystment process is usually accomplished in less than two hours, although it may extend over several. The shortest observed time is one hour and twenty minutes.

The position of Japanese varieties of Paramecium caudatum with respect to American varieties. LAUREN C. GILMAN.

The occurrence of ten varieties of two matings each in collections of *Paramecium caudatum* from various localities in the United States has been previously reported (*Biol. Bull.*, 97:239). In addition an eleventh variety which gives no reaction with the previous ten was found in collections made in New Haven, Connecticut in the summer of 1949. This variety was designated as variety 11 and the mating types present as XXI and XXII in accordance with the scheme used on the other varieties.

The presence of four non-interbreeding varieties of *P. caudatum* in collections from the Island of Honshu was reported by Hiwatashi (*Sci. Repts. of the Tohoku U.* 4sr. (Biol.) **18**: 137–140). Representative cultures of these four varieties were obtained from Hiwatashi in Sept., 1949 through the kind cooperation of the Medical Department of the Occupation Forces. Each variety consists of two interbreeding mating types as do the American varieties and were designated according to the same system.

Samples of each of the Japanese varieties were mixed with samples of each of the American varieties when, as the result of test mixtures with appropriate controls, all were known to be in good reactive condition. The mixtures were examined immediately for mating reactions and later for conjugation.

The results of the mixtures are shown below. Corresponding mating types in the two systems are found on corresponding lines. Where no corresponding variety was present in the American collections a new designation according to the American system was given.

Gilman's variety	System type	Hiwatashi's system	
		Variety	Type
1	I	1	1
	II		2
2	III		
	IV		
3	V	3	6
	VI		5
4	VII		
	VIII		
5	IX		
	X		
6	XI		
	XII		
7	XIII		
	XIV		
8	XV		
	XVI		
9	XVII		
	XVIII		
10	—		
	XX		
11	XXI		
	XXII		
12	XXIII	2	3
	XXIV		4
13	XXV	4	7
	XXVI		8

There are now known, then, thirteen varieties in *Paramecium caudatum*. Two of these, variety 1 and variety 3, are found in both the United States and Japan. Nine, varieties 2, 4, 5, 6, 7, 8, 9, 10, and 11 are so far found only in the United States. Two, varieties 12 and 13, are known so far only from Japan.

The marine sand dwelling Ciliates of Cape Cod shores. E. FAURÉ-FREMIET.

Two categories of Ciliates species dwell in the damp sand of marine beaches; some of these seem to be so closely adapted to interstitial conditions that they do not live outside; some

others enter the sand easily and can live in it as well as anywhere else along the seashore, where they are ordinarily widespread.

With regard to the size of the interstitial sand spaces, which are roughly proportional to the size of the grains, the sand dwelling Ciliates can be divided into two ecological classes: In the coarse sand, with a mean diameter of the grains above 0.4 mm., the "mesoporal" Infusorian fauna includes few species exclusively sand living, and many occasional sand dwelling ones. In fine sand, with a mean grain diameter of 0.3 to 0.7 mm., the "microporal" Infusorian fauna is composed of true sand-living Ciliates species. In addition, some typical interstitial species, widespread into fine and coarse sands, may be called "euryporal."

Comparison of the Ciliates interstitial fauna observed in Brittany and Cape Cod beaches shows that interstitial species are cosmopolitan and that the distinguishing characteristics of meso- and microporal fauna have a generalized ecological significance. On the other hand, it appears that the truly microporal species are endowed with some adaptive peculiarities very interesting from the standpoint of cytology, cell-physiology and micro-ecology.

Presence of Protohydra leukarti Greef at Barnstable Harbor. E. FAURÉ-FREMIET.

No abstract submitted.

Vascular patterns and behavior in typical parapodia of Nereis virens and Nereis limbata. PAUL A. NICOLL.[1]

Although activity of vascular systems is usually associated with mechanisms that circulate the blood or regulate pressure gradients, many observations indicate blood vessels may exhibit motor phenomena that serve some other purpose.

The remarkable alternation of contraction and relaxation, in a more or less rhythmical pattern, exhibited by arterioles and precapillary sphincters in many tissues of vertebrates, and by the veins in a few cases such as the wing membrane of bats, has been termed rhythmical vasomotion. This activity certainly is not directly involved in circulation of the blood, nor does it appear to modify any total pressure gradients within the system. It is rather an independent effector mechanism mediated by activity of individual muscle cells in the vessel wall, and in most instances is not primarily under control of the nervous system.

It was hoped that the great diversity of form of the vascular systems in the Polychaetes would make them a profitable group to study the nature of the behavior of such small vessels. However, it soon became evident that detailed studies on the vascular systems of the available Polychaetes were lacking. After a preliminary survey, work was concentrated on the parapodial circulation of *Nereis virens* and *Nereis limbata*.

The general characteristics of the vascular systems of the two forms are very similar. The classical description for *N. virens* by Turnbull, which has been followed by recent authors, is both incorrect and inadequate in many details. No segmentally arranged direct connections occur between the dorsal and ventral longitudinal vessels. Rather, three lateral segmental vessels are present that are functionally quite separate. One arises from the dorsal vessel; a second originates, at least functionally, from the intestinal capillary plexus; and the third arises from the ventral vessel. In *N. limbata* this third vessel has two important branches.

Space does not permit a description or discussion of these vessels or their activities. Each makes some anastomoses through the superficial capillary plexus with the others which allows some circulation through the parapoda. More striking, however, is the fact that to a large but variable extent, each vessel terminates in a rich arborization of blind-ending tubes. The high contractility of these blind tubes and their supplying vessels results chiefly in an ebb and flow of blood, rather than true circulation, that must produce local pressure changes similar to those developed in capillaries of vertebrates by rhythmical vasomotion.

Failure to find intercellular protoplasmic continuity in Griffithsia globulifera. HOWARD J. CURTIS AND JOHN L. MYERS.

It has been claimed by many investigators that there is protoplasmic continuity between adjacent cells in many plants, and the red marine alga, *Griffithsia globulifera*, is said to exhibit

[1] Supported in part by a U. S. Public Health grant.

this very conspicuously. Evidence for this concept has been almost entirely of a morphological nature, and the present work is an attempt to add evidence of a physiological nature. Strands of single cells of Griffithsia were isolated and placed in a recording chamber consisting of two electrode wells cut in a block of wax. The wells were filled with isotonic KCl or sea water with the cell-strand bridging the space between the wells, and the whole chamber was covered with a layer of mineral oil. There were usually about three cell-junctions between the wells. With KCl in one well and sea water in the other a substantial resting potential was observed only if a substantial fraction of one cell protruded from the KCl. As the strand was pushed over so that more of the cell was in KCl, the potential fell and became practically zero as the meniscus approached the cell junction. On pushing still further, the meniscus passed the junction so the KCl wet the next cell, and the potential increased again. The same results were obtained when both wells were filled with sea water and one end of a cell was mechanically injured. When several cells in a line were injured, the injury potentials would add to give a potential which was the sum of the individual injury potentials. The only reasonable explanation of these results would seem to be that there is a very high electrical resistance between the cytoplasm of adjacent cells, and thus, no protoplasmic continuity.

On the absence of a fine internal network in erythrocytes of Elasmobranchs. A. K. PARPART.

Erythrocytes from individuals of several species of Elasmobranchs (smooth dogfish, dusky shark, sting ray) have been examined by means of televised microscopy. The wavelengths of the light used for this study lay between 365 mμ and 420 mμ.

The red cells of blood freshly removed from these Elasmobranchs contain between 30 and 100 granules which are in active Brownian movement throughout the cytoplasm. These granules vary in size from 0.1 to 0.4 μ. The rapid movement of the granules throughout the cytoplasm, their continual collision with the cell and nuclear surfaces strongly suggest that not only is there an absence of a fine internal network in these red cells but that the hemoglobin in their interior is in true solution.

These granules in the red cells are probably the same type that Nittis and also Dawson brought out by vital staining.

The granules disappear from the red cells of blood that is allowed to stand at ca. 23°C. for 20 to 24 hours; but not if the blood is kept at 5°C.; nor if the cells are suspended in isotonic Na Cl and kept at 23°C. This suggests that the granules may be metabolites which are utilized by the cell and not replaced when the cell is kept *in vitro* in its plasma at 23°C. The granules are present in red cells that have been osmotically lysed, and show even more active Brownian motion. Dogfish red cells washed centrifugally three times with 0.33 *M* Na_2SO_4 undergo a loss of the nucleoprotein from their nuclei without loss of hemoglobin from their cytoplasm. Cells thus treated show no loss of granules, though the Brownian movement of the latter becomes less marked, owing, probably, to increased viscosity of the cytoplasm.

The role of epidermis in amphibian regeneration as revealed by triploid and haploid transplants to diploid limbs. ELIZABETH DEXTER HAY.

The role of the wound epithelium in the regeneration of amphibian limbs has been a controversial subject. Rose has suggested that the epidermis dedifferentiates to give cells for the early blastema. The following experiment was designed to test this hypothesis.

Heteroploid epidermal transplants were used as a means of tracing the individual epithelial cells. Haploid, diploid and triploid cells were identified by the number of nucleoli in the nucleus and by nuclear size.

Pieces of tailskin removed from triploid *Rana pipiens* tadpoles were transplanted to the thighs of stripped hindlimbs of diploid tadpoles. Tailskin transplants from haploid tadpoles were placed on diploid and triploid host limbs. Such transplants are almost entirely epithelium and have a very thick basement membrane. The opposite limbs were stripped as controls. Both limbs of each animal were amputated through the ankle at this time and the regenerates were fixed at various stages of growth. Histological sections were stained with Heidenhain's hematoxylin. This paper reports the positive findings up to this time.

The transplanted epidermis migrates as a sheet to the tip of the limb and covers the wound surface. The transplant's basement membrane remains on the thigh in the region where the strip of tailskin was placed. This was followed by means of haploid epidermal transplants identified by nuclear size. Both haploid and triploid epidermis piles up at the tip of the limb 5 days after amputation. At 6 days after amputation this epidermal cap disappears. It is not possible to follow the haploid any farther because cells cannot be identified by nuclear size once inside the blastema due to the tremendous range in cellular size there. However, triploid cells can be identified by the number of nucleoli. Diploid blastema cells never have three nucleoli. Numerous triploid and tetraploid mesenchymal-like cells have been found inside the blastema of a 6-day regenerating diploid limb which had received a triploid-tetraploid transplant. By present histological criteria these epidermal cells inside the blastema have dedifferentiated. We are particularly interested now in determining whether these undifferentiated blastema cells which have come from the epidermis redifferentiate into the adult tissues of the new limb.

Transphosphorylation in annelid muscle. WARREN H. YUDKIN.[1]

Arginine phosphate, present in the molluscs, arthropods, and to a certain extent in echinoderms, is replaced in the annelids by two as yet unidentified compounds with the chemical characteristics of phosphagens. One of these phosphagens found in annelids behaves on hydrolysis very much like creatine phosphate, and so it was believed, provisionally, to be identical with creatine phosphate by Baldwin and Yudkin (*Proc. Roy. Soc. London B*, 1950, **136**:614). In the present investigation this hypothesis was tested by determining the ability of crude extracts of body wall muscle of *Glycera dibranchiata* and *Neanthes virens* to phosphorylate creatine using adenosine triphosphate as a phosphate donor. Previously, Baldwin and Yudkin showed that *G. dibranchiata* contains only a creatine phosphate-like phosphagen and that *N. virens* contains both an arginine phosphate-like and a creatine phosphate-like phosphagen. Ochoa (*Biochem. J.*, 1938, **32**: 237), Lehmann (*Biochem. Ztschr.*, 1935, **281**: 271), Lohmann (*Biochem. Ztschr.*, 1936, **286**: 28), and Baldwin and Needham (*Proc. Roy. Soc. London B*, 1937, **122**: 197) have provided some evidence that the phosphokinases which are responsible for the transference of phosphate from adenosine triphosphate to arginine or creatine are specific for their respective guanidine bases.

The test system used in the present experiments contained adenosine triphosphate, creatine or arginine as phosphate acceptor, $MgCl_2$, veronal buffer at pH 7.85 and a KCl extract of muscle which had been dialyzed against KCl solution for 48 hours. In some experiments NaF was added or $MgCl_2$ was omitted. Determinations of acid-soluble phosphate were carried out after the mixtures had been allowed to incubate for 45 minutes at room temperature. Under these conditions it was readily demonstrated that frog (*Rana pipiens*) muscle phosphorylated creatine but not arginine and that scallop (*Pecten irradians*) muscle phosphorylated arginine but not creatine. Similarly prepared extracts from *G. dibranchiata* and *N. virens*, on the other hand, esterified neither creatine nor arginine. It is unlikely, therefore, that the creatine phosphate-like phosphagen present in these forms is identical with creatine phosphate.

Progress report on investigations of the Nudibranchiata of New England. GEORGE M. MOORE.

The author has undertaken a faunistic survey. Most information about New England nudibranchs comes from workers of the last century. Balch published a few records before 1910. It is apparent that the New England nudibranch fauna is not so varied, nor are the individuals so numerous, as on the coasts of the British Isles or on the Pacific coast of America. The following lists species found by the author chiefly from the intertidal zone, with dates and localities. Kodachrome photographs of living animals have been made of all except *Idulia* spp.

1. *Palio lessonii* (Orbigny). One from Rye, N. H., in tide pool, 7/22/50.

2. *Acanthodoris pilosa* (Abildguard) [= *Doris bifida* Verrill]. Very numerous on rocks and *Fucus* in intertidal zone at Dover Point, Great Bay, N. H., April and May, 1949 and 1950. A single specimen from the open coast at Kittery Point, Me.

[1] This work was supported by a grant from the Williams-Waterman Fund of the Research Corporation to Dr. George Wald.

3. *Onchidorus fusca* (Müller) [= *Doris bilamellata* L. of Alder and Hancock]. Not numerous but found in mature condition on rocks in tide pools throughout the year, in Great Bay, N. H. and on the open coast from York, Me., to Rye, N. H. Small immature specimens very numerous during late July and August, 1950, in same localities.

4. *Onchidorus aspera* (Alder and Hancock). Collected during fall, winter and spring on open coast from York, Me. to Rye, N. H.

5. *Dendronotus frondosus* (Ascanius) [= Dendronotus arborescens (Müller)]. On rocks and *Fucus* in intertidal zone in spring (few) and summer (numerous) in Great Bay, N. H., and on coast from York, Me. to Rye, N. H.

6. *Idulia coronata* (Gmelin) [= *Doto coronata* Gmelin]. At Newcastle, N. H. on *Sertularia* (7/26/50) and at Woods Hole, Mass. on *Tubularia* (8/16/50).

7. *Idulia pinnatifida* (Montagu). In Great Bay, N. H. on *Sertularia* (7/15/50).

8. *Coryphella rufibranchialis* (Johnston) } Most numerous of eolids collected. Abun-

9. *Coryphella rufibranchialis chocolata* Balch } dant on rocks in tidepools on open coast from York, Me. to Rye, N. H. from late April through June. Few in Great Bay, N. H. Balch's variety with dark ceratia composes about 25% of population. His dark variety intergrades with the red.

10. *Eubranchus pallidus* (Alder and Hancock) [= *Galvina picta* (A&H)]. Collected twice (York, Me. and Rye, N. H.) April, 1950.

11. *Tergipes despectus* (Johnston). On *Sertularia*, Newcastle, N. H. (8/10/50).

12. *Cratena pilata* (Gould). On Pennaria at Woods Hole, Mass., Aug., 1950.

13. *Cratena aurantia* (Alder and Hancock). On *Tubularia* at Woods Hole, Mass. (6/21/50 and 7/3/50), Isles of Shoals, Me. (7/25/50), Great Bay, N. H. (8/19/50).

14. *Aeolidia papillosa* (L.). Three specimens at Woods Hole, Mass. (June, 1949) and York, Me. (April, 1950).

PAPERS READ BY TITLE

The effect of Janus Green B on cleavage and protoplasmic viscosity. ROBERT DAY ALLEN.

Janus Green B (0.2 to 0.9 milligrams per cent) causes marked differential delay or even complete inhibition of cleavage in the eggs of the sea-urchin Arbacia, the sand-dollar Echinarachnius, the clam Mactra, and the worm Chaetopterus when applied three minutes (in Mactra, Arbacia and Echinarachnius) or five minutes (Chaetopterus) after fertilization. Earlier application of the dye usually caused total inhibition, whereas later application brought about little or no delay or inhibition. Cleavage was delayed in Arbacia eggs up to twenty-five minutes, in Echinarachnius fifteen, Mactra ten, and Chaetopterus less than ten. In Chaetopterus eggs there is a greater tendency toward inhibition rather than delay.

Fertilized eggs allowed to remain in low concentrations of the dye never passed the early gastrula stage, and usually stopped at the four or eight cell stage. Eggs fertilized in the dye seldom cleaved and were mostly abnormal. The inhibitory effects of Janus Green B were irreversible; inhibited eggs did not recover when returned to sea-water.

Janus Green is listed by Jorpes (1947) as an anticoagulant for blood. Consequently, protoplasmic viscosity determinations were made by the centrifuge method (Heilbrunn and Wilson, 1948) at 34, 38 and 40 minutes after fertilization at 21°C. Protoplasmic viscosity was still low at 34 and 38 minutes in the experimental eggs, although at this time the control eggs in seawater showed typical mitotic gelation. At 40 minutes gelation had begun in some of the experimental eggs.

Growth of pedigreed strains of Paramecium caudatum and P. aurelia on a nonliving medium. W. D. BURBANCK.

Using Gilman's *Paramecium caudatum* (Number I) and Sonneborn's *P. aurelia* (Stock 51), 3 day mass cultures were run with daily counts. Single washed animals (Parpart's Method) were introduced into 1.0 ml. of three different media. Hard glass watch glasses were used and kept in Petri dish moist chambers. All runs were maintained at 25.5 ± 0.5°C. Tests for contamination were negative.

Living *Aerobacter aerogenes* (Sonneborn's Number 41 Aa) grown on lettuce infusion served as food for control paramecia and killed, as one ingredient of food for ciliates grown in a non-living medium. For control animals, 24 hour *Aerobacter* was diluted one-half with distilled water. The two non-living media consisted of killed *Aerobacter* with equal parts of 0.5% Anheuser-Busch yeast autolysate. In one case (van Wagtendonk's medium) bacteria were killed by autoclaving at 15 pounds for 20 minutes, while in the other case, by subjecting them to 60°C. for 10 minutes.

The following results were obtained:

	No. of runs	Av. daily div.-rate	Highest daily div.-rate
Paramecium caudatum			
Control	8	0.5	1.0
In three of the eight runs there were no divisions. *Aerobacter* overgrew paramecia.			
van Wag.'s medium	14	0.3	0.6
In no case did the animals divide the first day. In three days, 3 divided twice, 5 once, and 6 survived but did not divide.			
Bacteria killed at 60°C	19	1.0	1.3
In all but two cases, animals divided the first day.			
Paramecium aurelia			
Control	8	2.7 plus	3.0 plus
van Wag.'s medium	16	0.9	1.3
Bacteria killed at 60°C	16	2.2	2.5

Although the lettuce-bacteria-autolysate medium is a complex one, division-rates obtained when bacteria were killed at 60°C. were quite constant and repeatable. Particularly in the case of *P. aurelia* sufficient numbers of animals were obtained in three days to provide material for a variety of experiments.

Phosphorus compounds in the sea urchin and starfish eggs. EDWARD L. CHAMBERS. No abstract submitted.

Enzymic determination of adenosine triphosphate in the unfertilized and fertilized egg of the starfish. EDWARD L. CHAMBERS. No abstract submitted.

Observations on maturation of starfish eggs in which the nucleolus has been removed from the germinal vesicle. EDWARD L. CHAMBERS. No abstract submitted.

Viability of Arbacia gametes in relation to time. RALPH HOLT CHENEY.

In an attempt to reduce the slaughter, which is a factor in the current scarcity of *Arbacia punctulata* in the Woods Hole region, several investigators (Palmer, 1937, *Physiol. Zool.*, **10**: 352; E. B. Harvey, 1939, *The Collecting Net*, **14**: 180; 1940, *Biol. Bull.*, **79**: 363; Pequegnat, 1948, *Biol. Bull.*, **95**: 69; Tyler, 1949, *The Collecting Net*, **19**: 19) have suggested ways and means, primarily through the injection of isosmotic KCl (0.5 Molar), of "sexing" the individuals as to their maturity, for selective storage, and for the shedding of successive batches of eggs and sperm over a period of six weeks without sacrificing the life of the animal.

It may be helpful to Arbacia investigators, and in the interest of conservation, to report that during the past several summers, incidental to a study of the life span of Arbacia gametes *per se* under different concentrations of certain drugs, it has been observed that the eggs and sperm can be utilized satisfactorily as "fresh" for at least an eight hour post-shedding period in the case of the eggs and for a minimum of a 24 hour time lapse in the case of the sperm. It is unnecessary to collect gametes from (or to sacrifice) additional animals for preliminary experiments and for most types of data with the exception of the final critical studies in respiration.

The following procedure provides both "fresh" eggs, as determined by their 100% fertilizability; and, "fresh" sperm, as determined by their ability to fertilize; and, by the normalcy of the fertilized egg to develop in normal time and form into the pluteus larva. Eggs or sperm were shed into stender dishes containing 40 cc. of sea-water, allowed to remain in a concentrated, undisturbed mass, and with the cover allowing a small air space, were placed so that running sea-water surrounded the dishes up to one-half their depth. For use, two cc. of sea-water eggs (taken each time from the edge of the concentrated mass shed) were mixed with two drops of sea-water sperm (likewise obtained from the concentrated mass shed) or one drop of "dry" sperm, in a stender dish containing 40 cc. of sea-water. This procedure results in the formation of the fertilization membrane and the development into the larva in normal time and form in 100% of the eggs under these conditions. They respond in a manner precisely equivalent to the results seen by mixing freshly-shed gametes.

Sampling eggs of various ages (subsequent to shedding from one female) were mixed immediately and after 1, 2, 4, 8, 12, 16, 20, 24, 30, 36, and 48 hours, with sperm of the same post-shedding age (obtained from one male); and, also separate eggs of these same ages were mixed with fresh sperm. Likewise, sperm of these same ages were employed to fertilize fresh eggs. Tabular expression of the experimental statistics is given below.

Viability time table for Arbacia gametes

Post-shed. age of sperm in hrs. fresh	Post-shedding age of egg in hours →							
	Immediate mixing of fresh egg and fresh sperm gives 100% fertiliz. membrane*	4-hr. egg ×fresh sperm results in 100% fertiliz. membrane	8	12	16	20	24	30
			100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
4	Fresh egg ×4-hr. sperm 100% F.M.	4-hr. egg ×4-hr. sperm 100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
8	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
12	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
16	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
20	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
24	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	80%	0–1% F.M.	No F.M.
30	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	75%	0–1% F.M.	No F.M.
36	75% F.M.	75% F.M.	75%	75%	75%	50%	0–1% F.M.	No F.M.
48	No F.M.	No F.M.	0%	0%	0%	0%	0%	0%

* F.M. formed typically and development occurs normally.

Results indicate: (1) The functionally normal, post-shedded life span of the unfertilized egg is appreciably less than that of the sperm. (2) For routine preliminary experiments, and with the exception of critical respiratory studies, eggs may safely be considered as well within the limits of dependability to function as physiologically "fresh" certainly for as long as eight hours and the sperm for twenty-four hours after shedding under the conditions of maintenance and use as stated above. One hundred per centum, or the equivalent of the control in any given batch of eggs, of the fertilizability of the eggs; and, of the fertilizing power (capacity) of the sperm, is retained during these periods and the subsequent development is normal in time and form.

The practice of the above procedure would enable zealous investigators, using large numbers of *Arbacia punctulata* gametes, to conserve their time and aid in the conservation of the species without jeopardizing the accuracy of their work. Similar conservation of other sea-urchin species may be worthy of consideration also by investigators at other Marine Laboratories.

The cytochrome oxidase and succinic dehydrogenase contents of squid (Loligo pealii) axoplasm and other nervous tissues. S. J. COOPERSTEIN AND ARNOLD LAZAROW.

Previous studies on dog nerve indicate that whereas the concentration of succinic dehydrogenase is below the sensitivity of the method used, measurable amounts of cytochrome oxidase are present. Since these two enzymes are almost invariably present together in tissues, we have studied their distribution in the giant axon and in other nervous tissues of the squid.

Cytochrome oxidase was measured spectophotometrically by following the rate of oxidation of reduced cytochrome *c* at 550 mμ. Succinic dehydrogenase was determined by following the rate of reduction of oxidized cytochrome *c* in the presence of sodium succinate and cyanide (Cooperstein *et al.*, 1950). The right giant axon was dissected and cleaned and a sample of axoplasm weighing between 2.00 and 5.00 mgs. was extruded and analyzed. The left giant axon was similarly dissected and the enzyme content of the whole nerve determined. Samples of fin nerve, stellate ganglion, muscle, and a composite sample of the fibrous sheath plus small nerves surrounding the giant axon were also studied. The enzyme activity was expressed as Δ log [cytochrome *c*] min./mg. wet weight of tissue. Five samples of fin nerve and 6–8 samples of the other tissues were analyzed.

Significant quantities of succinic dehydrogenase were found in squid axoplasm (.108) compared to dog nerve which contains $<$ .012 (limit of sensitivity). Similar concentrations were found in the intact giant axon (.120), fin nerve (.113), and fibrous sheaths plus small nerves (.112). Muscle and ganglion contain significantly greater amounts of this enzyme (.155 and .227, respectively).

The cytochrome oxidase content of axoplasm (.395) was the same as that of intact giant axon (.375), fin nerve (.360), and fibrous sheaths plus small nerves (.410), but significantly less than muscle (1.53), and ganglion (2.12).

The cytochrome oxidase content of squid stellate ganglion is over five times that of dog spinal ganglion; the activity of the giant squid axon is 3.6 times that of dog nerve. The succinic dehydrogenase content of squid stellate ganglion is 1.74 times that of dog spinal ganglion.

Comparative studies on the cytochrome oxidase and succinic dehydrogenase content of toadfish (Opsanus tau) and rat kidneys. S. J. COOPERSTEIN AND ARNOLD LAZAROW.

The cytochrome oxidase and succinic dehydrogenase contents of various tissues were determined spectrophotometrically by measuring the respective rates of oxidation and reduction of cytochrome *c* under standardized conditions. Samples of tissue were removed from 10 toadfish and 8–10 rats and the enzyme activities per mg. wet weight were compared for corresponding tissues.

In most tissues studied the ratio of the cytochrome oxidase concentration in the rat to that of the toadfish ranged from 0.97 to 1.66 (brain, 0.97; muscle, 1.06; liver, 1.38; and heart, 1.66). By contrast the cytochrome oxidase content of rat kidney was 3.13 times that of the toadfish.

Similarly, in the case of succinic dehydrogenase the ratio of enzyme concentration in rat tissue to that in toadfish ranged from 3.0 to 4.19 (brain, 3.00; heart, 3.03; muscle, 3.32; and liver, 4.19). On the other hand this ratio was 9.93 in kidney.

Thus the relative deficiency of these enzymes in toadfish kidney as compared with rat kidney is much greater than in other tissues. Whether this striking difference can be correlated with the aglomerular condition of toadfish kidney, with the marine habitat, or with the presence of gills which may take over a part of the renal excretory function, awaits further investigation.

Dividing Arbacia eggs as test objects for the effects of cortisone and desoxycorticosterone on cell division, potassium permeability, and glucose utilization. IVOR CORNMAN.[1]

Marine eggs would be a convenient tool for examination of the mechanism of adrenal steroid action, if the eggs could be made to exhibit any of the responses observed in mammalian systems. Division of Arbacia and Echinarachnius eggs is retarded by cortisone at 10–40 mg./L and desoxycorticosterone (DOC) at 5–10 mg./L. Both completely suppress Echinarachnius cleavage at 100 mg./L. DOC glucoside, although more soluble, is inactive at 1000 mg./L. Using concentrations at and below the slightly inhibitory level, the steroids were tested in situations with some resemblance to conditions under which they are active in the mammalian body. When Echinarachnius eggs are exposed to both steroids simultaneously, the delay is additive. The KCl-resistant and KCl-susceptible phases of the fertilized Arbacia egg (Chambers) are not altered by DOC, nor is the recovery in sea water following KCl-blocking of division. Thus DOC exhibits no tendency to permit K penetration during the resistant phase, does not prevent K penetration during the susceptible phase, and does not prevent K escape when the eggs are returned to sea water. Cortisone or DOC alone, glucose alone, or cortisone + glucose do not accelerate division or embryogenesis. An influence of glucose on the dividing egg can be manifested by means of colchicine, which becomes less inhibitory in the presence of glucose. This glucose effect is not enhanced by cortisone. The systems within the egg conceivably are remote from situations prevailing within the mammalian body. Therefore, the absence of demonstrable effects in echinoderm eggs sheds little light on the mechanism of action of the adrenal steroids.

Growth of Campanularia colonies. SEARS CROWELL AND MALCOLM RUSK.

Colonies of *Campanularia flexuosa* have been kept growing in the laboratory for several weeks by using the following procedure. A stem with from 10 to 25 hydranths, from a freshly collected colony, is tied by a thread to a glass slide. Such a stem develops attaching stolons in a day or two. From these stolons new stems (hydranths and hydrocauli) arise. The slide is placed in a rack in running, filtered sea water. Newly hatched nauplii of Artemia are fed to the specimens twice daily.

The average time required for the development of a new hydranth, once its production was started, was 24 hours at 20–22°C., and 37 hours at 17°C. However, colonies doubled their mass in 3½ days at 17°C. as against 6 days at 20–22°C. Thus more hydranths were produced at a time at the cooler temperature.

Gonangia were produced at 17°C. but not at 20–22°C. When a gonangium was produced there was nearly always a (compensatory?) regression and resorption of the hydranth nearest the developing gonangium.

If a colony can show regulative or compensatory growth when its typical composition is altered, one would expect that the production of new stolons and stems would be more rapid in a colony from which nearly all of these components had been removed. Conversely, terminal growth from stems would be more rapid when their tips had been removed, leaving a colony with an excessive stolon system. Amputated colonies showed no tendency toward regulation in the manner suggested. Growth of new structures went forward from each locus of growth just as if no alteration of the composition of the colony had been effected. This result parallels those of Berrill who finds that the nature of the structure produced is dependent on slight differences in the morphology of the loci where their proliferation begins.

Phosphatase activity in Tetrahymena. ALFRED M. ELLIOTT AND ROBERT L. HUNTER.

Information concerning the phosphatase activity among the protozoa is even more scanty than for the invertebrates in general. Kind (*Biol. Bull.*, 1949, **97**: 262) found a "progressive

[1] Aided by a grant from Bache Fund of the National Academy of Sciences and by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University.

increase in alkaline phosphatase activity with increasing biological complexity" among several marine invertebrates. It seemed important to examine these enzymes in at least one member of the first phylum of animals. *Tetrahymena geleii* was selected for investigation because its physiology and biochemistry is rather well known and its possibility as an experimental animal of importance shows promise.

When washed organisms (approximately 10,000/ml.) were suspended in distilled water containing 0.001 *M* adenylic and cytidylic acid (separately) at pH 6.0, 50% of the phosphorus was released into the supernatant in 45 hours of incubation at 24°C. Hence phosphatases are present in Tetrahymena.

Following the same procedure, but removing one-half the contents of the flask after 14 hours of incubation and filtering the protozoa, the phosphorus level remained constant in the filtrate, indicating that the enzymes were confined to the organism itself.

Similarly the pH optima were found to be approximately 5.2 and 8.7, being considerably more active in the acid range, which would be expected in view of Kind's observations on invertebrates.

By employing a synthetic medium (*Physiol. Zool.*, 1950, **23**: 85) which contained nucleic acid components as substrate and no inorganic phosphate added except that inadvertently included with protogen, it was found that phosphorus release proceeded rapidly prior to the onset of log growth, after which phosphate uptake obscured the picture. Total phosphorus readings of both supernatant and cells gave constant readings within an error of 5%. For several hours following log growth the organisms continued to take up phosphate at the same rate as during log growth.

"Spot" beats in deganglionated Limulus hearts. W. E. Garrey.

Removal of the median dorsal ganglionic cord stops the normal rhythmic beat of the heart of Limulus instantly and permanently. Twenty-four hearts were so prepared *in situ*. In 18 of these, subsequent examination revealed a few, small, discrete irregular areas of slow rhythmic contraction which were sharply localized; contractions which did not spread throughout the syncytial myocardium. Eleven of these contractile spots were located on either the first or second segment which are non-ganglionate. Heat applied to these spots did not accelerate their rhythm, the genesis of which, therefore, was not local or myal. A source of remote control was sought by exploring the dorsum of the posterior segments with a warm test tube. In 8 of the 11 instances, localized spots were found on the homolateral side of the posterior segment which accelerated the rhythm of the distant muscular contraction. The contractions stopped upon section of the fibers of the lateral nerve. A secondary extra-ganglionic neural mechanism is clearly involved in the "spot" contractions.

Microscopic examination of the localized neurogenis centers revealed small bipolar cells similar to those seen in the median ganglionic cord. No large, "pace-maker," nerve cells could be identified.

The effect of P^{32} on the division time of Arbacia eggs. J. W. Green and J. S. Roth.

Arbacia eggs were placed in sea water containing P^{32}. After an hour the eggs were fertilized and counts made, at intervals, of the first and second cleavages. The sea water was prepared by evaporating carrier free isotope solutions to dryness and then taking up the residue in sea water. Concentrations of 50, 5, and 0.5 μc of P^{32} per ml. were compared to an isotope free control. Preliminary results showed that the first and second cleavages were significantly delayed by the highest concentration of isotope. The intermediate and low isotope concentrations gave respectively smaller, but possibly significant, delays in both cleavages also. It is planned to extend the experiments by using different exposure times, concentrations and types of isotopes.

Staining procedures for whole mounts of the spinning glands of Habrobracon larvae. D. S. Grosch.

In an attempt to throw light upon the organization and nucleic acid relationships of the multinucleate units which comprise the spinning glands of the parasitic wasp Habrobracon (Grosch, 1950, *Proc. N. C. Academy of Science*), many of the stains in common usage have been employed. It was found that only by the Feulgen method (Rafalko modification) are

preparations produced which allow unobscured examination of nuclear elements. Stains such as haemalum and haematoxylin, commonly considered nuclear stains, are of little value because they give deep color to spinning gland cytoplasm. This disadvantage is found also for orcein and aceto-carmine; furthermore, technique which breaks the nucleus to release its contents is not recommended because of the large number of minute rod-shaped objects which resemble the chromosomes of a Hemipteran type somatic nucleus rather than the small number of large chromosomes of the Dipteran nucleus. An examination of slides containing series of glands from larvae of different ages stained with toluidine blue or Unna-Pappenheim pyronin-methyl green suggests that cytoplasmic basophilia increases during the four larval instars until the time of functional activity. This would indicate an increase in RNA during the development of the glands. Toluidine blue gives more repeatable results. The orange G component of Flemming's triple stain produces a very distinct histological picture of the arrangement of structural components, especially the trachea. Eosin was of little value.

Starvation studies with Habrobracon. II. Haploid males, diploid males, and diploid females compared in longevity. D. S. GROSCH AND A. M. CLARK.

Recently, using the two more common animal types, Grosch (*Biol. Bull.*, 1950) demonstrated that gentically diploid females survive the gentically haploid males under conditions of complete starvation. This difference was more pronounced at lower temperatures and there was a distinct positive correlation between wasp size and longevity. The present report extends these findings to the genetically diploid males. The three sex types (haploid males, diploid males, and diploid females) were obtained from a cross involving stock 25. Offspring 2.2 to 2.7 mm. in length were collected and stored one animal per vial. Survival time in hours, as given by the mean with its standard error, at incubator temperature (30°C.) was 147.20 ± 2.69 for haploid males, 134.30 ± 5.57 for diploid males, and 154.28 ± 2.78 for females; at room temperature (20–25°C.) survival time in the same terms was 181.20 ± 4.08 for haploid males, 177.84 ± 5.28 for diploid males, and 223.20 ± 3.84 for females. These results in which the diploid male is the least viable type indicate that genetic diploidy *per se* is not related to the longevity but that a sex difference is involved. This is consistent with the hypothesis that during starvation females have a somewhat different basis of metabolism associated with oosorption.

Variable sensitivity of Chaetopterus eggs to ultraviolet light. PAUL RANDOLPH GROSS.[1]

Sea-urchin eggs are more sensitive to ultraviolet light during the first 30–40 minutes after insemination than they are later in the mitotic cycle (Giese, 1947; Blum, 1950; etc.)

Fertilized eggs of the worm Chaetopterus were irradiated at various points in the mitotic cycle in order to determine whether these eggs also show variable sensitivity.

In these experiments, the source of radiation was the Westinghouse Sterilamp. The spectrum of this apparatus has a maximum at 2537 Å. A constant dose was employed in all experiments (3 minutes: 8640 ergs/mm.^{2}). With this equipment, little or no cytolysis was encountered, and the irradiated eggs ultimately cleaved in the same numbers as did the controls.

Assuming a cleavage time of 57 minutes for a typical control, the sensitivity varies thus:

The most sensitive part of the cycle lies in the time from fertilization to 30 minutes afterward. Our standard dose at any point in this period consistently induced cleavage delays of from four to eleven minutes.

The period from 32 to 40 minutes is relatively insensitive. Here the standard dose usually failed to produce any delay at all, and occasionally effected a delay of one or two minutes.

During the time from 40 to 50 minutes, an increase in sensitivity seems to take place. Irradiation in this period regularly produced cleavage delays of from three to six minutes.

We have not yet been able to obtain reliable values for the period from 50 minutes after fertilization to the time of cleavage.

These facts might suggest a correlation between the sensitivity to ultraviolet light and the viscosity changes demonstrated by Heilbrunn and Wilson (*Biol. Bull.*, 1948, **95**: 57), or they may be related to nuclear events which take place at the same time.

[1] Aided by a grant from the U. S. Public Health Service.

Photography of Arbacia punctulata eggs with infrared light. ETHEL BROWNE HARVEY AND GEORGE I. LAVIN.

Photographs of the stratified centrifuged egg of *Arbacia punctulata* taken with infrared light (8000–10,000A°) are similar to those taken with visible light, including the mitochondrial layer. The yolk granules, however, appear coarser and the red pigment granules appear similar to the yolk granules, not darker as with visible light. In photographs of the plutei also, taken with infrared, the red pigment spots, so characteristic with visible light do not show at all; otherwise the photographs with infrared and visible light are similar.

Photographs of the unfertilized Arbacia egg and of all stages of the fertilized whole egg and red "half-egg" have been taken with infrared light. The nuclei of resting stages, the streak of the very early prophase, the dumb-bell shaped mitotic figure of the metaphase, and the separated spheres of the telophase stand out with remarkable clearness as white areas in the dark granular-appearing egg. Cleavage planes are prominent as white lines. The nuclei of the many-celled early and late blastulae are so clear that they might readily be counted. A better configuration of the nuclear phenomena in the fertilized red "half-egg" is obtained than with visible light. The picture is not obscured by the red pigment granules which apparently do not absorb this wavelength of light. Owing to the penetrating power of infrared light, it is excellent for the general configuration of the mitotic figures, but owing to its poor resolving power, is not good for detailed structure.

Luminescent reactions in the "railroad worm," Phrixothrix. E. NEWTON HARVEY.

The luminous beetle, Phrixothrix, with both a red and yellow light organ is so rare that knowledge of its chemiluminescent processes must be gathered little by little. At the General Scientific Meeting in 1949, I reported that sodium adenosine triphosphate (ATP) does not revive the luminescence of extracts of either the red or the green light organs, a result contrary to the behavior of the fire-fly, *Photinis pyralis.* The experiment has been repeated and confirmed in 1950 on another specimen of Phrixothrix sent by Dr. P. Sawaya and the reason for the negative result discovered. Luciferin disappears quickly in the extract of Phrixothrix, for addition of purified fire-fly luciferin (kindly supplied by Dr. W. D. McElroy) to the extract of the yellow light organs containing ATP does excite luminescence. Adding fire-fly luciferin to the extract of the red light organ plus ATP gave only a very faint luminescence, if any.

A recent study of Jamacian fire-flies by McElroy and Harvey (to be published shortly) indicates three types. In some species (1), ATP disappears quickly in an extract of lanterns and light will reappear on adding ATP. In others (2) luciferin disappears quickly and no light can be obtained on adding ATP but luminescence will occur on adding luciferin. In still other species (3), luciferase disappears rapidly and neither ATP nor luciferin will restore the light but addition of luciferase from another fire-fly will do so. The yellow light organs of Phrixothrix appear to belong in the second category. Further work will be necessary to characterize the red luminescence. The red color of the light suggests oxidation of a porphyrin derivative rather than a compound similar to fire-fly luciferin. (See Harvey, *J. Cell. Comp. Physiol.*, 1944, **23**: 31; 1945, **26**: 185.)

Nitrogen deficiency and coloration in red algae. FRANCIS HAXO AND PHYLLIS STROUT.

Many of the Rhodophyta collected in the late summer from inshore waters are decidedly bleached in appearance. They may be variously almost colorless, pale red, tan, yellow, or green. When brought into the laboratory and maintained in tap sea water enriched with KNO_3, such thalli undergo a definite and in some cases striking color change, usually to deep red or brown, resulting from an increased synthesis of plastid pigments. All red algae tested showed a color change in varying degree. These were: *Chondrus crispus, Agardhiella tenera, Ceramium rubrum, Polysiphonia, Champia, Lomentaria, Ahnfeltia, Chondria* (3 species), *Porphyra variegata, Hypnea,* and *Gracilaria.* Control thalli kept in unenriched sea water retained their bleached appearance.

The minimal concentration of supplementary KNO_3 required to effect a reddening in *Agardhiella* and *Ceramium* lies between 0.01 mM and 0.05 mM. At higher concentrations up to 5 mM

a rather uniform reddening resulted, whereas 10 mM gave toxicity symptoms. The color changes were first noticeable within 24 hrs. and appeared complete within about 4 days.

The effect is specifically due to added nitrogen. KCl, phosphate, and ferric citrate were ineffective over a wide concentration range. NH_4Cl, $NaNO_2$, and NH_4NO_3 can each replace KNO_3 as sources of inorganic nitrogen for this reaction.

The photosynthetic rate of *Agardhiella* which had turned red under the influence of added nitrate was double that of a control portion of the same thallus kept in straight sea water. Development of fruiting structures (carposporangia) in *Agardhiella* and *Porphyra* was stimulated by supplementary nitrate.

The enzymes of the isolated islet tissue of the toadfish (Opsanus tau): cytochrome oxidase and succinic dehydrogenase. ARNOLD LAZAROW AND S. J. COOPERSTEIN.

It has been suggested (Lazarow, 1948) that the metabolic specialization of the beta cells for insulin synthesis predisposes these cells to the toxic action of alloxan. The reduction of alloxan and dehydro-ascorbic acid to dialuric acid and ascorbic acid respectively abolishes their diabetogenic effects. Since these compounds are reduced by certain enzyme systems while their reduced forms are reoxidized by oxidized-cytochrome *c*, we have studied the enzyme systems in the isolated islet tissue of fish.

The concentration of oxidized-cytochrome *c* in the cell is determined by the total cytochrome *c* present and by the enzymes which oxidize (cytochrome oxidase) and reduce (succinic dehydrogenase, etc.) cytochrome *c*. Cytochrome oxidase was measured spectrophotometrically by following the rate of oxidation of reduced cytochrome *c* at 550 mμ. Succinic dehydrogenase was determined by measuring the rate of reduction of oxidized cytochrome *c* in the presence of sodium succinate and cyanide (Cooperstein *et al.*, 1950). Samples of islet tissue weighing between 1.00 and 2.00 milligrams sufficed for the enzyme assay. The enzyme activity was expressed as Δ log [cytochrome *c*] /minute/mgm. wet weight of tissue and the average value for 10 animals was calculated.

The succinic dehydrogenase content of islet tissue (.085) was lower than that of brain (optic lobes) (.206), kidney (.239), liver (.412) and heart (1.373) but not significantly different from testis, gill, skeletal muscle, and ovary (.072–.085). The cytochrome oxidase content of islet tissue (.61) was significantly higher than that of muscle (.375) and gill (.465), but lower than that of ovary (.755), brain (.770), testis (.880), liver (1.98), kidney (2.02), and heart (3.50).

The ratio of succinic dehydrogenase to cytochrome oxidase in islet tissue (.139) was lower than gill (.170), muscle (.203), liver (.207), brain (.288) and heart (.392) and slightly higher than kidney (.119), testis (0.96), and ovary (0.96).

If the distribution of other cytochrome *c*-reducing enzymes parallels that of succinic dehydrogenase, these results would suggest the possibility that islet may have a high capacity for converting potential diabetogenic compounds into their active forms.

Are nutritive substances essential for hydranth formation in Tubularia? JAMES A. MILLER, JR.

The experiments of Barth (1938) and Rose and Rose (1941) showed that substances circulating in the coelenteron influence Tubularian reconstitution. Whether these substances are nutritive, inhibitory or both has not been fully answered.

The following experiments were designed to test whether there are nutritive substances circulating in the coelenteron which are essential for hydranth formation (Barth, 1938, 1940, 1944). The tips of eyedroppers, drawn to a diameter of about $\frac{1}{2}$ mm., were inserted into the proximal ends of Tubularian stem segments 10 mm. long. Twenty stems impaled as described were inserted into a battery jar 27 cm. in diameter through which there was a constant flow of filtered sea water.

The water in the eyedroppers was maintained about 5 cm. lower than that in the jar by inserting a small tube, connected with an aspirator, into the large part of the eyedropper. In this fashion a constant gentle flow of sea water was maintained through the ten experimental stems. Controls were treated similarly but were not connected with the aspirator. Patency of the experimental stems was determined at intervals by observing the rise of water in the eyedroppers when the suction was stopped for half an hour.

Experiment 1. One of the experimental stems became plugged and was discarded. So also were two in which the coenosarc was carried up into the eyedroppers. All of the seven remaining stems reconstituted (100%). 70% of the control stems reconstituted.

Experiment 2. Two experimental stems became plugged and two lost their coenosarcs. The six remaining all developed hydranths (100%) as compared with 80% of the controls.

Since the flow of water removed any nutritive substances which might have been released into the coelenteron and since the experimental stems reconstituted as well as did the controls, it is concluded that there are no circulating nutritive materials which are essential for hydranth formation.

Activation of Nereis eggs by a detergent. W. J. V. Osterhout.

Unfertilized eggs of *Nereis limbata* were exposed to 0.004 per cent duponol (chiefly sodium dodecyl sulfate) in sea water for 30 to 60 seconds. Jelly was extruded and in some cases segmentation occurred, and a small percentage of eggs formed trochophores which swam near the surface.

The procedure was as follows: Pasteurized sea water was used in all cases. The female was washed and was caused to shed in 250 ml. of sea water. The eggs were placed for 30 to 60 seconds in the solution of duponol in sea water and were then transferred to 250 ml. sea water where segmentation occurred in some cases. The temperature was approximately 25°C. Controls without duponol showed no segmentation.

No attempt was made to secure a larger percentage of trochophores since this was not the primary purpose of the experiments.

Relative solubility of the components of the Nereis egg. W. J. V. Osterhout.

The egg is surrounded by a tough vitelline membrane which is resistant to dissecting needles. It does not dissolve when exposed for several hours to 5 *M* H_2SO_4 but it dissolves in less than 5 minutes in 1% duponol (chiefly sodium dodecyl sulfate).

After the disappearance of the vitelline membrane the cytoplasm spreads out in the solutions and the nucleus is exposed to the action of the duponol. It does not dissolve but remains for several hours with little change in appearance except for slight swelling.

The oil drops show no change. The other constituents of the cytoplasm become dispersed in the surrounding solution and their fate cannot be precisely determined.

Javelle water is prepared from chlorinated lime in the usual way using sea water in place of distilled water. It is allowed to stand until a precipitate settles out and then diluted with 3 parts of sea water. This acts somewhat like 1% duponol except that the nucleus shrinks and becomes opaque.

With undiluted Javelle water everything dissolves except the oil drops.

A cytological analysis of the effects of cyanide and 4,6-dinitro-ortho-cresol on the mitotic phases in Arbacia punctulata. Allan Scott.[1]

It has long since been demonstrated by Clowes and Krahl (*J. Gen. Physiol.*, 1936, **20**: 145), by Robbie (*J. Cell. Comp. Physiol.*, 1946, **28**: 305) and by others that the oxygen consumption of fertilized Arbacia eggs is inhibited by suitable concentrations of cyanide and is stimulated by the nitrophenols. Moreover, both reagents suppress cell cleavage. We have treated the Arbacia eggs in sea water solutions of these inhibitors in concentrations ranging from 5×10^{-2} molar down to 10^{-5} molar (pH 8.1). In every case we have prepared slides to check the mitotic condition.

The first few minutes of development in all strengths of both reagents is at a nearly normal rate and this is true even when eggs are pretreated to insure penetration. Eggs pretreated for fifteen minutes with 5×10^{-2} molar NaCN or even for one hour in 10^{-3} NaCN, will fertilize in the solution, raise a fertilization membrane, show sperm penetration, male pronuclear migration and pronuclear fusion by plus fifteen minutes, but the male pronucleus fails to swell. If immersed at progressively later stages, development continues correspondingly longer but we find

[1] This study was made during tenure of a fellowship from The National Institute of Health, U. S. Public Health Service.

no phase specific inhibition. Eggs treated with high concentrations in the early prophase never emerge from the prophase simply because it is a phase of long duration. In 5×10^{-2} molar NaCN and in 10^{-2} molar 4,6-dinitro-ortho-cresol, there is little development beyond the first fifteen minutes, which is at the near normal rate. In lesser strengths development continues very slowly. Thus eggs immersed in 10^{-4} NaCN at thirty minutes gave no cleavage by 142 minutes, but 53% by 5½ hours. Robbie found a maximum inhibition of oxygen uptake at about 10^{-4} molar NaCN.

Eggs in 10^{-4} 4,6-dinitro-ortho-cresol at thirty minutes (crescent stage) arrive at prometaphase at fifty minutes, scarcely delayed over metaphase controls. At seventy minutes there are a few anaphases, at 107 minutes a few are cleaved and at 280 minutes: 20% are one cell, 58% are two cell, and 22% are three or four cell. Clowes and Krahl found that the optimum stimulation of oxygen consumption was close to 10^{-6} molar for this reagent. In 100 × this concentration we find slow development during 4½ hours.

Factors involved in the breakdown of the germinal vesicle in the egg of Chaetopterus pergamentaceus. ALLAN SCOTT AND GEORGE LEBARON, JR.

The development of the Chaetopterus egg is arrested at two stages; the first arrest is at the germinal vesicle stage and the second, at the first polar metaphase which persists normally until fertilization. We are here concerned with the factors responsible for the first arrest.

The passage to the first metaphase is perfectly normal in many different solutions. It is normal, for example, in 2 × sea water, in 50% sea water and in calcium free sea water. It is unaffected by isotonic KCl, $CaCl_2$ or NH_4Cl. The pH range is very wide, for eggs will go to the metaphase in sea water at pH 5.0 and at pH 9.0. Transfer to viscous solutions of ½% agar in sea water or 6% egg albumin (dialized against sea water) does not affect the movement to the metaphase, indeed the process is uninhibited in mineral oil. It is markedly slowed, but not inhibited by sea water at 7°C.; however, one minute at 42°C. inhibits germinal vesicle breakdown completely. Ninety-two per cent of the eggs of some females, ovulated into parapodial fluid, may remain in the germinal vesicle stage for at least 60 minutes. This figure may be as low as thirty-two per cent in other females.

The breakdown of the germinal vesicle is completely inhibited by strong NaCN (.05 *M*), and the reaction is reversible since eggs treated for as long as an hour will give good cleavage. Similarly there is a persistent germinal vesicle in .05 *M* sodium azide and in sea water saturated with H_2S. There is no inhibition by carbon monoxide in the light or in the dark, nor is the process inhibited by .05 *M* iodoacetic acid.

We feel that this development to the first polar metaphase resembles parthenogenetic activation in that a delicately poised reaction is triggered by many types of treatment including pressure. Apparently cytochrome oxidase is not involved in the mechanism and it remains to be seen what enzymes are being inhibited by the cyanide, azide and H_2S.

The influence of the glycolytic inhibitor iodoacetic acid on aging and on the potassium and sodium content of the egg cells of Mactra solidissima. GEORGE T. SCOTT AND HUGH R. HAYWARD.[1]

The egg cells of Mactra undergo marked and peculiar morphological changes on aging over a twenty-four hour period. At about twelve hours they undergo a deep tubular indentation and assume a cup-like shape. At twenty to twenty-four hours a membrane is raised from the egg, and later the germinal vesicle disintegrates. Up to this time, according to Schechter (*J. E. Zool.*, 1941, **86**: 461), the eggs are fertilizable.

Associated with the aging process is a loss of potassuim and an uptake of sodium by the cells. The potassium begins to leave the cells at the time the indentation phenomenon becomes pronounced (9–12 hours), and concomitantly the sodium enters. At 24 hours, 70% of the potassium has escaped and the sodium content has more than doubled. These changes are described in the following table:

[1] Aided by a grant from the Biochemical Section of the Office of Naval Research with Oberlin College.

Age in hours	K meq. %	Na meq. %	% Indentation	% Raised membranes
2	12.7	14.6	0	0
4	12.5	19.4	0	0
6	13.1	16.0	4–5	0
9	13.1	15.9	10–12	0
12	9.1	22.7	40	0
15	6.0	25.2	15	85
23	3.6	38.3	0	90–95

The action of 0.001 *M* iodoacetic acid is such as to retard the development of the morphological aging signs and to cause the cell to maintain the original electrolyte levels for a longer time. The potassium then leaves the cell at a slower rate and the rate of entrance of sodium is also reduced. At about 36 hours the potassium and sodium content of the cells in iodoacetic acid resembles that of the controls. The action of the inhibitor on these cells is the direct opposite of the heretofore reported studies of its influence on potassium retention. This inhibitor causes a loss of potassium from erythrocytes, Arbacia eggs, brain cortex and yeast, presumably because of the inhibition of underlying glycolytic metabolism. It is possible that the slowing down of some metabolic mechanism by the inhibitor in the Mactra egg may retard the "aging" process.

Aqueous chlorophyll preparations from blue-green algae. JULIUS SILBERGER AND FRANCIS HAXO.

Tolypothrix lanata, Scytonema crispum, and *Oscillatoria* yield aqueous whole pigment extracts which are quite clear and give sharp absorption spectra similar to that reported by Emerson and Lewis (*J.G.P.*, 1942, **25**: 579) for *Chroococcus.* Addition of 15% $(NH_4)_2SO_4$ to the fluorescent extracts causes separation of a green precipitate after several hours. Most of the precipitate can be readily resuspended in water or pH 7 phosphate buffer to give green, nonfluorescent but slightly opalescent "solutions." Such initial preparations contain bound chlorophyll and carotenoids essentially free from phycocyanin.

Preliminary attempts have been made to purify and characterize the aqueous chlorophyll fraction from *Tolypothrix.* Crude extracts prepared by grinding or blending were cleared by filtration and low speed centrifugation. Fractional centrifugation between 2000 and 140,000 × gravity yielded a series of progressively smaller sediments, the major portion being thrown down below 20,000 g. The supernatant contained c-phycocyanin in solution. Absorption spectra of fractions obtained between 2000 and 80,000 g indicated uniform pigment composition. The curves are sharp and mainly characteristic of chlorophyll-a with the peaks displaced about 13 mμ toward longer wavelengths from those in methanol.

Addition of acetone or methanol to the resuspended green sediment caused flocculation of a colorless material which gave positive biuret and ninhydrin reactions for proteins. The extracted fat-soluble pigment consisted of chlorophyll-a and predominantly one epiphasic carotenoid. The suspensions are not coagulated by heat but show markedly altered absorption in the visible spectrum, the peaks shifting to shorter wavelengths.

It seems probable that such aqueous extracts contain chlorophyll-a and carotenoids bound together on the same particles, perhaps protein in nature, and not in true solution as is phycocyanin. Further studies of these preparations including their photochemical activity are planned.

A study of the development of the fin and trunk musculature in Elasmobranch fishes. WILLIAM L. STRAUS, JR.

Following the investigations of Balfour and others in the last quarter of the past century, it was generally accepted that in all vertebrates the striated musculature of both trunk and limbs is derived ontogenetically from the somites alone. Recent studies, experimental and otherwise, have demonstrated that the musculature of the extremities (possibly excepting some limb-girdle muscles) is of nonsomitic origin in tetrapod vertebrates (amphibians, birds, mammals; reptiles?), developing *in situ* from the limb-bud mesoderm. The view persists, however, that the

entire trunk musculature is formed by the somites alone. Recent experimental studies on the chick (*Anat. Rec.*, 1948, **100**: 755; complete report in preparation) demonstrate that whereas the intrinsic back muscles are of somitic origin, the abdominal (ventrolateral) trunk muscles arise directly from the non-segmented mesoderm of the lateral plate. Experiments on larvae of the urodele amphibian, *Amblystoma* (Straus, unpublished), suggest similar origins of these muscles.

The classical studies of the development of the limb and trunk musculature of elasmobranch fishes were made by Balfour and others. The results of these studies, carried out entirely on normal fixed and stained specimens, has obviously influenced subsequent thinking. From the published work on elasmobranchs, it appears probable, although not certain, that the muscles of both the paired fins and the trunk are of somitic origin. If this is true, the development of the striated musculature differs among the several classes of vertebrates.

With this possibility in mind, the writer has been collecting normal developmental series of both the skate (*Raia erinacea*) and the smooth dogfish (*Mustelus canis*), with the view of re-studying the normal development of the musculature of the paired fins and trunk in elasmobranchs and comparing it with muscular development in other vertebrates. Such a re-investigation is clearly indicated.

An even more conclusive approach to the problem would be an experimental one, such as has been made in the chick and amphibians. The writer has, therefore, been studying living embryos of the skate toward this end. The reproductive habits of the skate would seem to make this animal a particularly favorable one for such studies (as by carbon marking and extirpation of somites, etc.). The inability at present to secure prolonged survival of the embryos (necessary because of the very long period of development in all elasmobranchs) has proved to be an obstacle to the application of experimental methods; but it is believed that this obstacle is not unsurmountable.

Studies of the carbohydrate metabolism of invertebrate tissues in vitro. CLAUDE VILLEE, ROBERT LICHTENSTEIN, NEAL NATHANSON AND BRITA ROLANDER.

A study was made of carbohydrate metabolism in tissues from certain marine animals cultured *in vitro*, to determine whether the utilization of glucose and the synthesis of glycogen could be demonstrated and whether insulin had any effect on these processes. The tissues, thin sheets of gill or muscle or slices cut with a Stadie microtome, were incubated in Warburg vessels in artificial sea water (Van't Hoff B with calcium) containing 200 mg. per cent glucose at 25 ± 1°C. As shown in the Table, glucose uptake and glycogen synthesis occurred in all tissues tested but the addition of insulin (0.5 unit/ml.) had no effect in increasing these processes in any save skeletal (branchial) muscle from the dogfish, in which a threefold increase was obtained. No differences were observed in dogfish muscle incubated in isosmolar mixtures of artificial sea water, glucose, and urea in concentrations of 0, 2.3, or 4.7 gm./100 ml. These results confirm the work of Florkin and Duchateau (1939) who were unable to demonstrate any *in vivo* effect of insulin on the blood sugar content of crayfish.

Tissue	Number of determinations	Initial glycogen*	Glycogen synthesized†	Glucose uptake†	Oxygen uptake†
Mactra muscle slices	5	.82	20.0	—	3.5
Mactra gill	15	.40	3.3	4.2	11.3
Pecten gill	10	.14	2.3	6.4	9.9
Pecten mantle muscle	5	.25	1.1	1.2	5.8
Mya gill	10	.29	2.3	2.8	12.5
Busycon white muscle slices	10	3.55	12.2	2.3	2.3
Busycon red muscle	2	1.76	—	—	—
Chaetopterus muscle	10	1.03	1.8	11.8	5.2
Thyone muscle	30	.61	2.6	4.7	1.4
Dogfish branchial muscle	14	.30	1.8	3.0	2.3
Dogfish smooth muscle	6	.18	1.8	—	4.0

* The figures given are mg. glycogen per 100 mg. (wet weight) tissue.
† The figures given are micromoles per hour per gm. (wet weight) tissue.

Phosphorus metabolism in embryonic and adult invertebrate tissues. CLAUDE VILLEE, ROBERT LICHTENSTEIN, NEAL NATHANSON AND BRITA ROLANDER.

Studies of phosphorus metabolism in embryonic and adult invertebrate tissues were made by culturing fertilized eggs and excised adult tissues in sea water plus radioactive phosphorus (P^{32}), added as inorganic phosphate. Fertilized eggs of the sand dollar, Echinarachnius and the sea urchin, Arbacia, were used. The adult tissues used were gills from the clams Mya and Mactra and the scallop Pecten. After incubation the tissues were fractionated by a modified Schmidt and Thannhauser method and the specific activities of acid soluble P, phospholipid P, total acid insoluble P, desoxypentose nucleic acid P, pentose nucleic acid P, and phosphoprotein P were determined. The order of activity in embryonic tissue was DNA > PNA > phosphoprotein, as in previous studies (Villee *et al.*, 1949). The order in adult tissues was phosphoprotein P > PNA > DNA, phosphoprotein phosphorus being turned over 10 to 40 times as fast as the phosphorus in DNA. The amounts of phosphorus in the several fractions in the adult tissues remained essentially constant over incubation periods up to twenty hours. Adult tissue has a large fraction (about 40%) of the total acid insoluble phosphorus present as DNA P, and this P is turned over only very slowly. In contrast, developing embryos have only a small fraction of the total acid insoluble P (1.5% in unfertilized eggs, 11% after 10 hours of development in Echinarachnius) present as DNA P, but this P is turned over quite rapidly.

The occurrence of a new variety containing two opposite mating types of Paramecium calkinsi as found in sea water of high salinity content. RALPH WICHTERMAN.[1]

Enormous numbers of *Paramecium calkinsi* repeatedly were found living and reproducing in pools of sea water of high salinity content ("evaporation pools") near the sea in the region of the sand dunes of West Barnstable, Massachusetts. Described previously from fresh and brackish water, this represents the first account of this species from this type of environment. The salt water in which the paramecia flourished showed the salinity to be 37.66 (as determined by the Woods Hole Oceanographic Institution); sea water around Woods Hole has a salinity of 31–32. Even in water of such great salt concentration, the paramecia possessed contractile vacuoles. Single specimens and conjugating pairs in the original fluid swim characteristically and conspicuously in right spirals, the only species of the genus to show this feature.

While the paramecia were cultivated successfully in an infusion made of desiccated lettuce and filtered sea water only, maximal growth was obtained by using two parts of standard lettuce infusion in distilled water and one part filtered sea water.

Since many conjugating pairs were found in the original collections, single specimens were isolated and many clonal cultures established to test for mating. All possible mixtures were made not only among the newly established clones but among others maintained in permanent culture for several years including the two Yale clones of opposite mating type. Up to the present time, only this one variety with its two opposite mating types, has been described for this species. The result of all possible mixtures of the newly established clones and Yale clones resulted in the discovery of a second variety containing two opposite mating types. As of now, there exists two non-interbreeding varieties each containing two opposite mating types.

A simple method for obtaining large numbers of fission-stages of Paramecium and certain other negatively geotropic ciliates. RALPH WICHTERMAN.[2]

By means of a new device, it is possible to obtain quickly and easily large numbers of concentrated dividing stages of Paramecium and certain other negatively geotropic ciliates. The method is based on the fact that dividing specimens not only move more slowly than typical vegetative specimens but once division has progressed to the point where a definite transverse constriction furrow is formed, the fission stages, especially late ones with their characteristically uncoordinated manner of swimming, tend to settle toward the bottom of the culture container.

[1] Aided by a grant from the Committee on Research and Publication, Temple University.

[2] For aid in the construction of the device, acknowledgment is gratefully made to Mr. James G. Graham, Jr., Mr. Michael Troisi and the Committee on Research and Publication, Temple University.

The device consists of a 4000 ml. Pyrex beaker from which the bottom has been cut off and ground smooth. To this opening is fused a Pyrex funnel with a 60° angle and of the same diameter as the opening at the bottom of the cut beaker. To the stem of the funnel, there is joined the inner member of a 19/38 standard taper, ground-glass joint while the outer member is fastened to a glass stopcock having a 5 mm. opening. The ground-glass joint is held firmly in place with two coiled springs. The glass device is set on a stand consisting of a wooden base and upright steel arm which holds two adjustable, rubber-covered brass rings. The smaller ring supports the funnel section and the larger ring supports the beaker section.

The device is used in the following manner: a rich culture (approximately 4 liters or less) of species pure *Paramecium caudatum* is placed in the unit. At the peak of the logarithmic growth phase of the culture after the paramecia are supplied with ample bacterial food, three or more generations per day are produced. By simply opening the stopcock from time to time and drawing off a few cc. of culture fluid, great numbers of fission-stages may be obtained in fairly concentrated condition.

It should prove of value to those who require concentrated numbers of division stages in studies dealing with cytology, irradiation and action of chemicals.

Proteolytic enzymes in the eggs of the clam, Mactra solidissima. A. A. WOODWARD.

Intracellular proteinases are being investigated in the effort to elucidate the mechanism of protoplasmic clotting. In this study the eggs of the large clam, *Mactra solidissima*, were used. Eggs taken from the dissected ovaries were repeatedly washed to eliminate the possibility of contamination by alimentary proteases. The tough outer membranes were softened by treatment with isotonic urea, and the eggs were then washed and homogenized in a medium containing 0.34 *M* potassium chloride, 0.105 *M* potassium citrate, 0.053 *M* sodium chloride, buffered to pH 8.0 with 0.005 *M* imidazole. The hemoglobin method of Anson (1938) was used to assay homogenates or their fractions for proteinase activity.

A graph of pH against enzyme activity reveals prominent proteinase activities with pH optima at pH 7.5–7.6 (Enz I) and at pH 3.5–4.0 (Enz II). Preliminary experiments indicate that Enz I is activated by Ca ions. Enz II is apparently not affected by Ca ions.

The method of centrifugal fractionation was used to achieve a degree of separation of the various protoplasmic particulates. Four reasonably distinct fractions may be obtained: (1) Large granules, easily resolvable under the microscope (234 × g for 10 min.); (2) intermediate granules (234 to 446 × g for 30 min.); (3) small granules (25,000 × g for 90 min.), unresolvable or invisible under the microscope; and (4) granule-free supernate.

Fractions (1) and (2), tentatively identified as yolk platelets and mitochondria, respectively, have negligible proteolytic activity. Enz I activity appears in fraction (3), small granules, and in fraction (4), clear supernate; fraction (3) has greater specific enzymatic activity but less total amount of activity than fraction 4. Enz II activity is almost entirely associated with fraction (4), the clear supernate.

Water and biological morphology. DOROTHY WRINCH.[1]

The fundamental importance of water throughout biological studies needs little emphasis and it is to be anticipated that water molecules play a decisive role in all living systems. Gradually accumulating experimental data from various fields offer suggestions as to what this role may be.

The morphological potentialities of the HOH molecule are demonstrated by the structure analyses of ice. Each HOH molecule in such crystals surrounds itself with 4 of its fellows which it holds by means of OH..O hydrogen bridges at definite distances in definitely related directions—at the vertices of a regular tetrahedron, at distance 2.7A. The long known tridymite-like ice has hexagonal symmetry. In the recently discovered cristobalite-like ice (*Z. f. Krist.*, 1944, **105**: 279) the HOH molecules again array themselves tetrahedrally, but now with cubic (T_d) symmetry, a fact of great moment in connection with protein structure, since a close relationship between skeletons of protein molecules and cubic (T_d) networks has been surmised (*Biol. Bull.*, 1945, **89**: 192). It is also known from the case of (*e.g.*) oxalic acid dihydrate that

[1] This work is supported by the Office of Naval Research under contract N8onr-579.

individual water molecules can clamp other molecules together in hydrogen bridge circuits. Particularly interesting are the 5- and 29-hydrates of phosphotungstic acid in which not only individual waters but "crystal" fragments such as crumpled hexagons and even massive water clusters of considerable size and precise (actually cubic T_d) architecture function in the same way. The precise interpretation of the numbers of water molecules per anion which result ($5 = \frac{1}{2}(6+4)$, $29 = 1+4+6+6+12$) is a beautiful example of the impact of number theory on a structure problem in precisely the manner in which it may be expected also to uncover the significance of particular proportions of individual amino acids which have been found in certain proteins.

The body of results, of which examples have been given, make it highly probable that water molecules play a definitely structural role in living organisms. When the water complements are relatively low, they will undoubtedly act as clamps which hold other molecules in definite orientations, at definite distances apart. When, however, there are vast complements of water, we may anticipate also the formation of clusters of the most precise and varied morphologies. Just as in ice where these small molecules are capable of maintaining a precise alignment over macroscopic dimensions, so too in cytoplasm they may play a large part in holding other molecules and ions, and even massive protein structures, in structurally significant arrangements, extending over a complete living cell.

Water and biological function. DOROTHY WRINCH.[1]

A succinct demonstration of the functional role of water in living systems has recently been recorded (*Nature*, 1950, **165**: 938). A culture of encysted young *Amoeba proteus* was allowed to dry up completely until "nothing was left except a thin layer of greenish dust." Water was added and five months later young amoebae were observed and "it became clear that the culture was re-establishing itself." In the results obtained in x-ray crystal studies of proteins (*Cold Spring Harb. Sym. Quant. Biol.*, 1949, **14**: 65) and physico-chemical investigations (Svedberg and Pedersen, *The Ultracentrifuge*, 1940) we may find suggestions as to the interpretation of these facts.

Crystal studies of several proteins permit the following inferences to be drawn. Some proteins can crystallize with grossly different complements of water, with the molecular skeletons of the interlocked $NC_\alpha C$ monomers remaining intact: however, it is only in the presence of a sufficient complement of water molecules that the proteins can achieve the orderly setting of the R-substituents of their monomers in the definite and specific spatial patterns in which their physico-chemical personality and so, also their functional potentialities, inhere. Physico-chemical studies indicate that many proteins have particle rather than molecular status, being dissociable into sub-units, which also have the protein character. That water can dissociate proteins is known from the case of horse hemoglobin which dissociates into pairs of sub-units simply on dilution. But the physico-chemical individualities of protein particles necessarily depend upon the inter- as well as the intra-sub-unit patterns of the atoms. Hence a second way in which water molecules can affect the activities of proteins is suggested.

Extrapolating from cases such as these, we can now visualize how water plays an essential role in biological function, as for example in the "re-activation" of the *Amoeba proteus* culture. Only by dissociating the dried mass of protein structures from one another are these structures able to reassume their physico-chemical individualities and perform their biological functions. The way in which a physico-chemical stimulus, the addition of water, is able to initiate vast changes is then seen to depend upon the fact that the activities of proteins depend upon their physico-chemical individualities not merely on their chemical structures. In "dry" insulin crystals, as in "wet" insulin crystals, definite structures, which we may identify with the skeletons of the molecules, remain unchanged. However, it is only in the "wet" crystals that any orderliness from unit cell to unit cell can be imputed to the R-group atoms; it is only in the "wet" crystal, therefore, that the protein structures achieve orderly arrangements of skeletons *and* substituents. After the same manner, we may visualize the proteins with intact chemical structures even in the "thin layer of greenish dust" and understand how the reactivation of the proteins, which underlies the reactivation of the culture, is effected by the water molecules, which enable the proteins to reassume their physico-chemical individualities.

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REPORTS ON LALOR FELLOWSHIP RESEARCH

Studies on the distribution of the oxidative pathway of glucose-6-phosphate utilization. SEYMOUR S. COHEN.

Enzymes catalyzing the oxidation of glucose-6-phosphate and 6-phosphogluconate had been obtained from yeast, mammalian tissues, and *E. coli.* In recent studies in my laboratory it was shown that ribose-5-phosphate and triose phosphate was produced from 6-phosphogluconate in the enzymatic degradation of this substrate by cell-free preparations from yeast (*Science,* 1950, **111**: 543).

It has also been shown with *E. coli* that in adaptive growth on gluconate, the adaptive transphosphorylase, gluconokinase, is produced, converting gluconate to 6-phosphogluconate, used solely by the oxidative pathway. Nevertheless this partial bypass of the Meyerhof scheme permitted normal growth of the bacteria (*Nature,* In press).

It appeared of interest to see how widespread these dehydrogenases are from the point of view of assessing the general importance of this oxidative pathway, and secondly of determining whether the essential metabolite, ribose-5-phosphate, may be generated from other pathways. A spectrophotometric test was used for assaying homogenates of various organisms for the presence of dehydrogenases for glucose-6-phosphate and 6-phosphogluconate. The reduction of the coenzyme, triphosphopyridine nucleotide (TPN) was followed at 340 mμ as the reaction progressed in the absence of an autoxidizable carrier.

Both enzymes were found in homogenates of Chaetopterus sperm, the blue-green alga, *Tolypothrix lanata,* the red alga *Ceramium rubrum,* the green alga *Ulva lactuca,* and of frog heart.

Several attempts were made to demonstrate the enzymes in the protozoan, *Tetrahymena gelii,* and a brown alga, *Ascophyllum,* without success. The possible significance of negative results of this type will be discussed. However, the wide distribution of the enzymes is of interest.

Studies on spindle material. J. F. DANIELLI.

Experiments on the isolation of spindle material from the first division of the ovum of marine organisms were performed. After fertilization, the fertilization membranes are removed and the eggs allowed to swell to bursting in solutions having an osmotic pressure 10–30% that of sea water. Solutions containing mixtures of potassium and magnesium are preferable for this purpose. By this procedure, spindle material is obtained which is swollen and distorted.

Auxin effects in Bryopsis. WM. P. JACOBS.

Although auxin, the plant growth-hormone, has been shown to be present in many algae, including *Bryopsis,* its role in algae is still unknown. Since the most striking effect of auxin in *flowering* plants is the induction of roots, experiments were designed to see if auxin would induce on the alga *Bryopsis* the algal equivalent of roots known as rhizoids.

Bryopsis plants were grown in a constant temperature bath (21.5 ± 1°C.) in a diffusion gradient of indole-acetic acid, a synthetic auxin. The highest concentration of auxin was at the basal end of the plant. A striking difference was shown between the treated plants and the controls: the great majority of side-branches of plants in the auxin-gradient formed rhizoids at their bases, while no rhizoids were formed by any of the side-branches of any of the control plants.

Three replications of the experiment gave similar results.

A further effect of the auxin-gradient was found. Inverted *Bryopsis* plants with the highest auxin concentration at the original base showed nullification or reversal of the normal tropisms. The rhizoids, which are normally positively geotropic, grew away from the pull of gravity and toward the higher auxin concentration. The side-branches, positively phototropic in the controls, continued growing at a 45° downward angle in the treated plants. These changed tropiotic

reactions are in agreement with the hypothesis that tropisms in *Bryopsis* are, like those of flowering plants, controlled by differential distribution and sensitivity to auxin.

A number of regeneration experiments were also tried with successful results.

The results relating to rhizoid formation and tropisms provide the first evidence that the ubiquitously occurring auxin has very similar effects in both algae and flowering plants.

Respiration and iodine uptake by the brown algae. SALLY KELLY.

The brown algae are noted for their capacity to accumulate iodine from dilute amounts in sea water. In experiments designed to demonstrate whether this property resembles that of salt accumulation by cells of higher plants, in being governed by respiratory mechanisms, the respiration of the brown algae, in particular *Ascophyllum nodosum* (Linnaeus) Le Jolis, and its effect on the uptake of radioactive iodine from sea water were examined.

The age of the tissue, thickness of segments and pH of the medium were among the factors which influenced the endogenous respiratory rate. Among the compounds which stimulated respiration were glucose (0.001 *M*), sucrose (0.01 to 0.05 *M*), malic, fumaric and succinic acids (0.025 *M*) and pyruvic acid (0.0025 *M*). Fermentation also was increased in the presence of glucose, pyruvic acid and malic acid. Respiration was inhibited by potassium cyanide (0.0001 *M*), sodium azide (0.001 *M*), iodoacetic acid (0.01 *M*), malonic acid (0.005 *M*), and potassium fluoride (0.025 *M*). Mannitol, a storage product of the brown algae, and catechol, had no stimulating effects. The inhibitors, fluoride and malonic acid, and the organic acid substrates were ineffective at the pH of the control medium, 6.5; their effects were observed, however, when the pH was lowered to 4.5, suggesting that the penetration of these compounds is an important factor in determining their influence on respiration.

The uptake of potassium iodide in concentrations of 0.05 to 0.1 mg./l (the concentration of iodine in sea water), tagged with the radioactive isotope, I^{131}, was influenced by such factors as age of the tissue and pH of the medium. The rate of uptake during the first hour of exposure to the radioactive solutions was rapid and fell off gradually during succeeding hours. The uptake by the algae during the experimental periods was accompanied by a corresponding decrease in the radioactivity of the solutions. Of the compounds which influenced respiration, glucose and sucrose (0.025 *M*) increased the amount of radioactive iodine taken up. Effective inhibitors were malonic acid (0.025 *M*), sodium azide (0.0001 *M*), idoacetic acid (0.0001 *M*) and potassium cyanide (0.005 *M*).

These results suggest that the respiratory mechanisms of the brown algae resemble those known for higher plant cells and that the phenomenon of iodine uptake by the algae requires energy of respiration. Whether the energy source comes chiefly from glycolytic or from oxidative processes, however, will require further study.

The effect of divalent ions on Echinoderm phosphatases. C. ALBERT KIND.

Tissue homogenates of many marine invertebrates have been shown to exhibit glycerophosphatase activity. With the exception of the Porifera, which appear to possess only an acid phosphatase, the pH-activity curves of these preparations show maxima at pH 4.5–5.0 and 8.0–8.5 and thus resemble the vertebrate phosphatases in this respect. In the interest of demonstrating an even closer relationship between the invertebrate and vertebrate enzymes a preliminary investigation has been made of the effects of magnesium, manganese, and cobalt ions on the alkaline phosphatases of the brittle-star *Ophioderma brevispinum* and the sea cucumber, *Thyone briareus*. The ability of these ions to activate the alkaline phosphatases of vertebrate tissues is well known. Recent interest in beryllium as an inhibitor of alkaline phosphatase suggested investigation of the effect of beryllium on the echinoderm enzymes.

The enzyme preparations used in the present study consisted of 4% homogenates of the central disk of *Ophioderma* and of the digestive tract of *Thyone*. Incubation with β-glycerophosphate was at 25°C. for 24 hours at pH 8.6. The metals were added as the chlorides and the concentrations given represent concentration in the incubation mixture.

Beryllium reduces phosphatase activity of both preparations in concentration as low as 10^{-6}M. At a concentration of 10^{-4}M the inhibition is about 40% and complete inhibition occurs at 10^{-3}M. Beryllium inhibition at 10^{-4}M is antagonized by the addition of either 10^{-4}M manganese

or 10^{-4}M cobalt, but magnesium is without effect. This antagonism of beryllium inhibition is similar to that described by Dubois *et al.* (*Science*, 1949, **110**: 420) for rat serum phosphatase.

The dialyzed homogenates can be activated by the addition of magnesium, manganese, or cobalt; maximum activation being obtained at concentrations of 10^{-1}M, 10^{-5}M, and 10^{-2}M, respectively.

In general the effects of divalent cations on the alkaline phosphatases of these two echinoderms resemble very closely the effects on vertebrate phosphatase. These observations suggest that the mechanisms involved in these effects are also comparable.

Reactions related to photosynthesis and respiration. ALAN H. MEHLER.

Since Hill demonstrated that isolated chloroplasts could utilize light energy to reduce certain iron salts and liberate molecular oxygen, there have been many investigations into the nature of this reaction as a part of the mechanism of photosynthesis. The ability of spinach chloroplasts to react with a variety of oxidants had been explored previously. In order to determine whether the pattern obtained was characteristic of all chloroplasts, attempts were made to obtain active cell-free preparations from different plants. No sustained activity was found in chloroplast preparations from the green algae *Vaucheria*, *Nitella*, *Cladophora*, and *Ulva*, from two species of *Equisetum*, or from duckweed, *Lemna minor*. An extract of *Tolypothrix*, a blue-green alga, evolved some gas when illuminated with quinone, and experiments are in progress to isolate the active component.

Clendenning recently found that *Chlorella* acts like chloroplasts in evolving oxygen when illuminated in the presence of Hill oxidants, and that the ability to carry out photosynthesis and respiration is irreversibly lost in the process. The effect of quinone is markedly different with different algae. *Ulva*, like *Chlorella*, carries out a typical Hill reaction, and has both respiration and photosynthesis completely inhibited. With *Tolypothrix* there is an incomplete Hill reaction, photosynthesis is inhibited, but respiration is not affected. The photosynthesis of *Dazya*, a red alga, is inhibited by quinone, but no Hill reaction is seen. Instead, the effect of light is to increase the rate of respiration, and the increase is maintained in the dark, similar to the increase obtained in respiration of cell-free preparations of spinach.

The identification of a typical oxidative enzyme, malic dehydrogenase, in extracts of *Ceramium rubrum*, *Ulva*, and *Tolypothrix* indicates that these various algae probably use a respiratory mechanism very similar to those of animals and other aerobic organisms. In the first two species isocitric dehydrogenase was also demonstrated.

Separation and characterization of neurosecretory material from Fundulus heteroclitus. STUART W. SMITH.

Scharrer and others have established the cytologically based concept of secretory activity of certain hypothalamic nerve cells in many, but not all, vertebrates examined. It is believed that the neurosecretory colloid is transported along the axons of these cells to the *pars nervosa hypophysis*. Melville and Hare demonstrated that the supraoptic region contains extractable materials with antidiuretic activity. On the basis of unpublished cytological evidence from material stained by the chrome alum-hematoxylin technique, shown by Bargmann to be specific for neurosecretory material, I am led to believe that hypothalamic neurosecretory material is probably uniformly present in all vertebrates, and that it is probably identical with the *pars nervosa* hormone(s). However, no one has thus far reported direct chemical or physiological evidence to prove the latter thesis.

It was the purpose of this work to separate morphologically intact neurosecretory material from hypothalami of *Fundulus heteroclitus* by fractional centrifugation, and to test the separated material chemically and physiologically. For each methodological trial 25–50 hypothalami, carefully freed of hypophysial tissue, were obtained from decapitated male animals and chilled to 0–4°C. Two homogenizing methods and many suspending media (Kassel's, Kallman's, and Van't Hoff's solutions; 0.88, 2.00, and 2.50 *M* sucrose, and 1.5 *M* NaCl) as well as many combinations of centrifugal force and time were tried. Temperature during centrifugation was 0–5°C. Results were controlled by examination of smeared centrifugates stained with chrome alum-hematoxylin-phloxin.

At the time of writing this report, it has not been possible to obtain an essentially pure preparation of neurosecretory droplets. However, concentration of intact droplets by a factor of approximately 100 has been achieved. The nucleoproteins of persistently contaminating nuclei have been eliminated by homogenization in 10 volumes of 1.5 *M* NaCl followed by storage in the cold for 12–16 hours prior to centrifugation.

Studies of effects of the concentrates on blood pressure of Fundulus have not been technically possible thus far. Antidiuretic activity in euryhaline teleosts is studied only with the greatest difficulty. A large supply of frozen, concentrated neurosecretion is being collected, therefore, to be used later in testing for pressor effects in a larger teleost and water balance activity in toads, as well as for attempts at further purification.

During the course of the work described, I have also collected fixed material from marine vertebrates to supplement a cytological study of neurosecretory phenomena in the sympathetic ganglion cells of higher vertebrates.